

REVIEW ARTICLE

Role of FGF/FGFR signaling in skeletal development and homeostasis: learning from mouse models

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Fibroblast growth factor (FGF)/fibroblast growth factor receptor (FGFR) signaling plays essential roles in bone development and diseases. Missense mutations in FGFs and FGFRs in humans can cause various congenital bone diseases, including chondrodysplasia syndromes, craniosynostosis syndromes and syndromes with dysregulated phosphate metabolism. FGF/FGFR signaling is also an important pathway involved in the maintenance of adult bone homeostasis. Multiple kinds of mouse models, mimicking human skeleton diseases caused by missense mutations in FGFs and FGFRs, have been established by knock-in/out and transgenic technologies. These genetically modified mice provide good models for studying the role of FGF/FGFR signaling in skeleton development and homeostasis. In this review, we summarize the mouse models of FGF signaling-related skeleton diseases and recent progresses regarding the molecular mechanisms, underlying the role of FGFs/FGFRs in the regulation of bone development and homeostasis. This review also provides a perspective view on future works to explore the roles of FGF signaling in skeletal development and homeostasis.

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INTRODUCTION

Skeletons are formed through two distinct developmental modes, namely intramembranous ossification and endochondral ossification. The former is directly accomplished by osteoblast differentiation from mesenchymal cells; the latter involves initial differentiation of mesenchymal cells into chondrocytes to form a cartilage template and subsequent replacement by bone.¹ The cranium and medial clavicles are formed through intramembranous ossification. Long bones, including the appendicular skeleton, facial bones and vertebrae, are formed through endochondral ossification.^{2–3}

Various signaling molecules control the process of skeleton development, such as fibroblast growth factor (FGF), wntless-type MMTV integration site family members (Wnt) and bone morphogenetic protein (BMP) signaling pathways. Among these signaling pathways, FGF/fibroblast growth factor receptor (FGFR) signaling is very essential. The 22 members of the FGF family mediate their cellular responses by binding to FGFRs. There are four distinct FGF receptors with differential FGF-binding properties.^{4–5} A typical FGFR contains an extracellular ligand-binding domain, a transmembrane region and an intracellular

divided tyrosine kinase domain. FGFs bind to the extracellular domain of FGFRs and induce the phosphorylation of tyrosine residues in the intracellular domain of FGFRs. The activated FGFRs recruits target proteins to its cytoplasmic tail and modifies these proteins by phosphorylation,⁶ leading to the activation of intracellular downstream signaling pathways, such as mitogen-activated protein kinase (Ras/MAPK), phosphoinositide 3-kinase/Akt (also known as protein kinase B), phospholipase C and protein kinase C pathways. Furthermore, FGF signaling can also stimulate the signal transducers and activators of transcription (STAT) 1/p21 pathway^{2,7} (Figure 1). Multiple kinds of mouse models with genetic modifications of FGF/FGFR have been generated. In our review, we summarize the use of these mouse models in the research of the role of FGF/FGFR signaling in skeleton development and homeostasis.

ROLE OF FGFRs IN BONE GENETIC DISEASES AND HOMEOSTASIS

FGFR1

FGFR1 is first expressed in the early limb bud.^{8–10} At the epiphyseal growth plate, FGFR1 is expressed in perichondrium,

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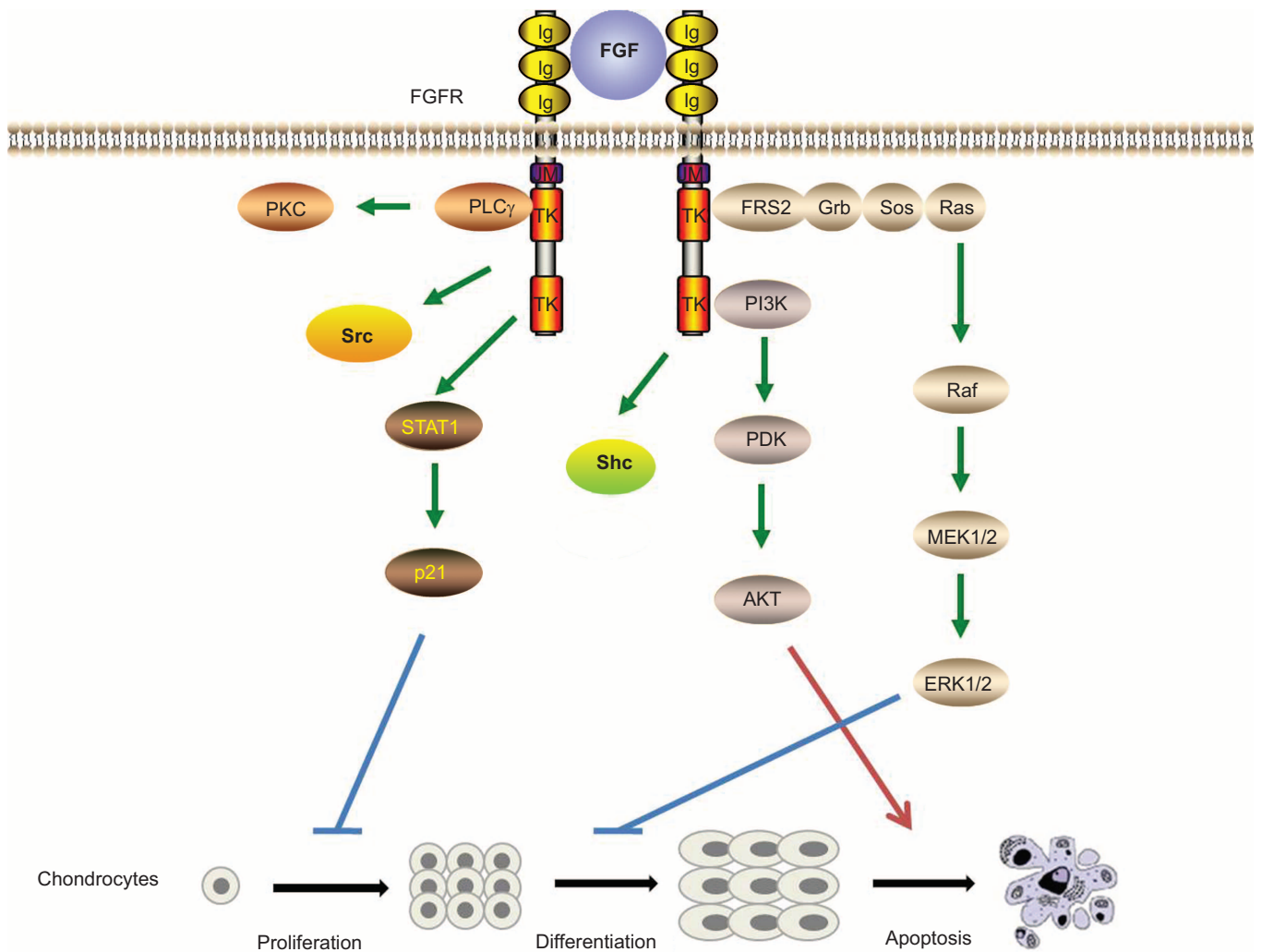


Figure 1. Signaling pathways activated by FGF/FGFR. FGFs induce dimerization, kinase activation and transphosphorylation of tyrosine residues of FGFRs, leading to activation of downstream signaling pathways. Multiple pathways are stimulated by FGF/FGFR signaling such as Ras-MAP kinase, PI-3 kinase/AKT and PLC- γ pathways. Furthermore, FGF signaling can also stimulate STAT1/p21 pathway. FGF/FGFR signaling also phosphorylates the Shc and Src protein. FGF/FGFR play crucial roles in the regulation of proliferation, differentiation and apoptosis of chondrocytes via downstream signaling pathways.

prehypertrophic and hypertrophic chondrocytes.^{9,11–12} FGFR1 is also expressed in osteoblasts and osteocytes (Table 1).^{13–16}

A series of mouse models of *Fgfr1* have been generated to genetically dissect the functions of *Fgfr1* during gastrulation and later developmental processes. *Fgfr1*-deficient (*Fgfr1*^{−/−}) embryos display severe growth retardation, and died prior to or during gastrulation because of intrinsic blocks in mesodermal differentiation.^{17–18} Deletion of the Ig domain IIIc of *Fgfr1* (*Fgfr1*IIIc) leads to gastrulation defects resembling the *Fgfr1*^{−/−} alleles. However, mice with *Fgfr1*IIIb ablation are viable and fertile, suggesting that IIIc is the dominant isoform for the majority of FGFR1 functions in embryogenesis.¹⁹ Chimeras were generated by injecting *Fgfr1*^{−/−} embryonic stem cells into wild-type blastocysts to circumvent the gastrulation defect. The

milder mutant chimeras exhibit deformed limb buds and varying degrees of reduction in limb skeletal elements.^{19–21}

Mice with targeted deletion of FGFR1 in all limb bud mesenchymal cells (via *T (brachyury)-cre*),²² or posterior limb bud mesenchyme (via *Shh-cre*)²³ were used to further study the role of FGFR1 in limb development. *T-cre; Fgfr1* mice die at birth and show reduced limb skeleton, misshapen forelimb/hindlimb bud and missing digits, whereas *Shh-cre Fgfr1* mice display normal limb bud size, but missed a digit.¹⁰ Li et al.²⁴ assessed the roles of FGFR1 signaling in forelimb and hindlimb development by disrupting this gene, using *AP2-Cre* and *Hoxb6-Cre* transgenic mice that express Cre recombinase in complementary temporal and spatial patterns during limb bud formation. The results indicate that disruption of *Fgfr1* at an earlier stage, prior to thickening of limb mesenchyme, results in

Table 1. The expression patterns of FGFs/FGFRs during skeleton development.^{2,7,11,31,46,60,161–162,204,262–263}

FGFs/ FGFRs	Limb bud	Osteoblast lineage	Cartilage	Cranial bone	Receptor specificity
FGF2	Developing condensation	Periosteal cells, Osteoblasts in trabecular bone	Perichondrium, Chondrocytes	Mesenchymal cells in the suture	FGFR1, FGFR2, FGFR3c, FGFR4
FGF4	Posterior AER at E10.5–11.0			Sutural mesenchyme in early craniofacial skeletogenesis	FGFR1c, FGFR2c, FGFR3c
FGF7	Loose mesenchyme		Perichondrium		FGFR2b
FGF8	AER	Cortical bone at embryonic stage	Perichondrium, Chondrocytes	Osteoblasts	FGFR2c, FGFR3c, FGFR4
FGF9	AER, Developing condensation	Periosteum, Primary spongiosa	Perichondrium, Chondrocyte primordia	Mesenchyme of suture in early craniofacial development stages	FGFR2c, FGFR3, FGFR4
FGF10	Lateral plate mesoderm				FGFR2b
FGF18	Perichondrium and presumptive joint positions		Chondrocytes,	Mesenchymal cells in the suture separating the two osteogenic fronts	FGFR2c, FGFR3c
FGF21			Chondrocytes		FGFR1–4
FGF23		Osteoblasts, Osteocytes	Resting and hypertrophic zone		FGFR1, FGFR3c, FGFR4
FGFR1	Mesenchyme (IIIc)	Osteoblasts in trabecular bone, Osteocytes	Prehypertrophic and hypertrophic chondrocytes of growth plate, Perichondrium, Cartilage of the cranial base	Dura mater and periosteum, Calvarial mesenchyme and later in osteoblasts	
FGFR2	AER (IIIb), Early limb bud mesenchyme (IIIc)	Periosteum, Trabecular bone (IIIc), Osteocytes	Prechondrogenic condensation, Resting zone of growth plate, Perichondrium, Cartilage of the cranial base	Proliferating osteoprogenitor cells and differentiating osteoblasts	
FGFR3	Center of the mesenchyme condensation	Osteoblasts, Osteocytes	Resting zone and proliferating chondrocytes of growth plate, Cartilage of the cranial base	Low levels in sutural osteogenic fronts at late stages of development	
FGFR4		Strictly in osteoblasts between the periosteal and endosteal layers	Resting and proliferative zones of growth plate		

more severe defects, characterized by malformation of the apical ectodermal ridge (AER).

FGF receptor-specific substrates (Frs) act as the principal mediators for FGFR1 signal transduction. Mice that lack the Frs-binding site on FGFR1 (*Fgfr1*^{ΔFrs/ΔFrs}) die during late embryogenesis, and exhibit defects in neural tube closure, and in the development of the tail bud and pharyngeal arches. However, mutant FGFR1 still has functions during gastrulation and somitogenesis, indicating that distinct signal transduction mechanisms of FGFR1 signaling in different developmental contexts.²⁵

Osteoglophonic dysplasia (OD) patients, resulting from activating mutations of FGFR1, exhibit rhizomelic dwarfism,²⁶ indicating that FGFR1 is a negative regulator of long bone growth. Embryos with conditional deletion of *Fgfr1* in osteochondro-progenitor cell lineages show increased height of the hypertrophic zone due to delayed degradation, or maturation of hypertrophic chondrocytes, or decreased osteoclastogenesis.¹⁵

Studies in humans and mice also reveal that FGFR1 play crucial role in bone formation. A gain-of-function mutation in FGFR1 (P252R) leads to Pfeiffer syndrome (PS), one type of craniosynostoses, characterized by premature fusion of one or several calvarial sutures.²⁷ Several activating mutations of FGFR1 in OD patients also lead to craniosynostosis

in addition to rhizomelic dwarfism.²⁶ Mice carrying a P250R mutation in FGFR1 were generated to mimic human PS. Studies using these mutant mice uncovered that FGFs/FGFR1 signals may regulate intramembranous bone formation.²⁸

Jacob *et al.*¹⁵ found that adult mice, with deletion of *Fgfr1*, exhibited increased bone mass. Deletion of *Fgfr1*, in osteochondro-progenitor cells in mice (via *Col2-cre*), leads to increased proliferation and delayed differentiation, and matrix mineralization of osteoblasts, while inactivation of *Fgfr1* in differentiated osteoblasts (via *Col1-cre*) causes accelerated osteoblast mineralization differentiation.¹⁵ It has been proposed that FGFR1 promotes the differentiation of mesenchymal progenitors into preosteoblasts, but inhibits the proliferation of mesenchymal progenitor cells, as well as the maturation and mineralization of osteoblasts.¹⁵ Impaired osteoclast activity is another reason for increased bone mass in mice with *Fgfr1*-deficient in differentiated osteoblasts. To explore the direct effect of FGFR1 on osteoclasts, Lu *et al.*²⁹ generated mice with targeted deletion of *Fgfr1* in bone marrow monocytes and osteoclasts using *LysM-cre*. The mutant mice exhibit increased bone mass, impaired osteoclast formation and activity indicating the positive regulation of FGFR1 on osteoclasts. The role of FGFR1 in osteocytes is still not

clarified and should be studied by deletion of *Fgfr1* in osteocytes using dentin matrix protein-1(*Dmp1*)-Cre (Figure 2).

In addition to the effect of FGFR1 on limb development and bone formation or remodeling, FGFR1 also participates in phosphorus metabolism. Osteoglophonic dysplasia patients have non-ossifying bone lesions, hypophosphatemia and increased serum level of FGF23, a member of the FGF family, which is a circulating phosphaturic hormone produced mainly by osteoblasts and osteocytes.^{30–31} Pharmacological inhibition of FGFR1 inhibits FGF23 transcription in bone of animal models.³² Integrative nuclear FGFR1 can activate the transcription factor cyclic AMP response element-binding protein (CREB),³³ which also binds the proximal *Fgf23* promoter; thus, it is hypothesized that FGFR1 may regulate FGF23 by binding CREB.

FGFR2

FGFR2 is expressed in condensing mesenchyme of early limb bud,^{9,34–35} and later appears as the marker of pre-chondrogenic condensations. In developing bone, FGFR2 is predominantly localized to perichondrial and periosteal tissue, and weakly to endosteal tissue and trabecular bone.³⁶ FGFR2 is intensely expressed in the cartilage of the cranial base and growth plate.^{11,37–40} In cranial sutures, FGFR2 is mainly expressed in osteoprogenitor cells¹³ and differentiating osteoblasts.^{41–42} The expression pattern of FGFR2 indicates its important role in skeleton development (Table 1).

Mice with deletion of transmembrane domain and part of kinase I domain of *Fgfr2* (*Fgfr2*^{−/−}) die at E4.5–5.5 due to stopped inner cell mass growth.⁴³ Targeted deletion of the Ig domain III of FGFR2 results in embryonic lethality at E10–11 because of failures in the formation of functional placenta. Mutant embryos also fail to form limb buds completely, indicating that FGFR2 Ig domain III is essential for limb initiation.^{24,44–45}

Activating FGFR2 mutations have variable effects on cranial cell replication, or differentiation in mice and

humans.^{40,46} More than 10 gain-of-function mutations in FGFR2 cause multiple types of craniosynostoses, such as Apert syndrome (AS), Crouzon syndrome (CS) and PS, as well as Beare–Stevenson cutis gyrata syndrome (BSS).^{40,47} Among them, AS is one of the most severe craniosynostoses. S252W and P253R mutations in FGFR2 are responsible for nearly all known cases of AS.^{2,47}

Several gain-of-function mutant mouse models, mimicking human craniosynostoses, have been generated to study the mechanism of FGFR2 for regulating the suture development. *Fgfr2*^{+/S252W} mutant mice mimicking human AS have smaller body size, midline sutural defect and craniosynostosis with abnormal osteoblastic proliferation and differentiation.⁴⁸ *Fgfr2*^{+/S252W} mice also show ectopic cartilage at the midline sagittal suture, increased cartilage in the basicranium, nasal turbinates and trachea. These mutant mice display long bone abnormalities, as evidenced by the disorganized growth plates and more prominent cartilage mineralization.⁴⁸ *Fgfr2*^{+/P253R} mice have growth retardation of the synchondroses of cranial base and growth plates of the long bones with decreased proliferation of chondrocytes, which may be responsible for the smaller body size and shortened cranial base in *Fgfr2*^{+/P253R} mice.³⁹ Furthermore, *Fgfr2*^{+/P253R} mice also show ectopic cartilages in the sagittal sutures^{39,49} consistent with the skull phenotypes in *Fgfr2*^{+/S252W} mice and the symptom in AS patients.^{48,50} However, Chen *et al.*⁵¹ found that the growth plates of *Fgfr2*^{+/S250W} mice showed slightly shorter columns of proliferating chondrocytes, but no abnormal hypertrophic zone; and premature closure of cranial base synchondrosis have been detected in *Fgfr2*^{+/S250W} mice.

In *Fgfr2*^{+/P253R} mice or *Fgfr2*^{+/Y394C} mice mimicking human BSS (also characterized by skull abnormalities), the premature fusion of coronal suture is associated with enhanced osteoblast differentiation similar to *Fgfr2*^{+/S252W} mice.^{39,49,52} In another mouse model with a C342Y mutation in FGFR2IIc (*Fgfr2c*^{+/C342Y}) (equivalent to mutation in human causes CS/PS), premature fusion of

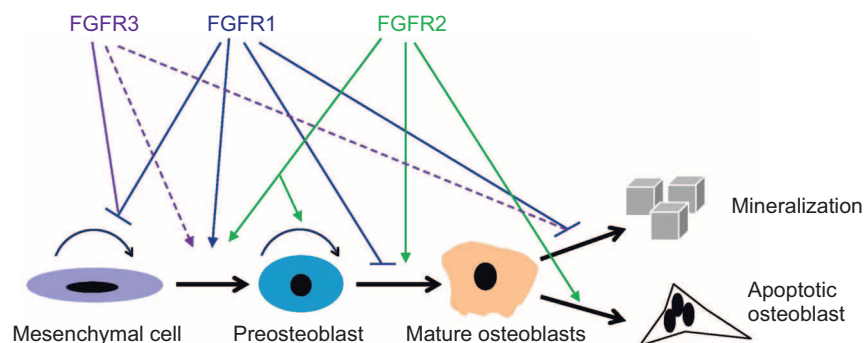


Figure 2. The regulation of osteogenesis by FGFR1-3. FGFRs play distinct roles during the differentiation of osteoblast. There are some conflicting results about the effect of FGFR3 on osteogenesis, which is marked by dotted lines.

cranial sutures is accompanied by enhanced osteogenesis and increased proliferation of osteoprogenitor cells in the coronal sutures.⁵³ Chen *et al.*⁵¹ also found decreased bone formation and premature closure of the coronal suture in *Fgfr2*^{+/S250W} mice similar to phenotypes in human AS.⁵¹ However, increased apoptosis is responsible for premature fusion in *Fgfr2*^{+/S250W} coronal suture.⁵¹ These results suggest that different activating mutations in FGFR2 result in craniosynostosis through distinct mechanisms.

Fgfr2^{IIIc}^{-/-} mice also show delayed differentiation and mineralization of the skull vault, and premature coronal suture due to decreased cell proliferation.⁵⁴ The retarded ossification in *Fgfr2*^{IIIc}^{-/-} mice is correlated with the decreased osteoblast markers *OP* and *Cbfa1*, which is emphasized by increased osteogenesis of Crouzon-like mutant *Fgfr2*^{+/C342Y} mice with upregulated *OP* and *Cbfa1* levels.⁵⁵ These results suggest that FGFR2IIIc is a positive regulator of intramembranous ossification. Conditional deletion of *Fgfr2* in mesenchymal condensations of mice via *Dermol-Cre* results in skeletal dwarfism and decreased bone density. The proliferation of osteoprogenitors and the function of mature osteoblasts are impaired in mutant mice. However, the differentiation of osteoblast lineage in mutant mice is not disturbed,³⁶ which is distinct from the delayed differentiation in *Fgfr2*^{IIIc}^{-/-} mice.⁵⁴ This finding may reflect the differences in the timing of *Fgfr2* inactivation.³⁶ These differences can be further explored by conditional deletion of FGFR2 in osteoblastic cells.

Fgfr2^{IIIc}^{-/-} mice also exhibit dwarfisms, reduced growth of the skull base and axial, as well as appendicular skeletons, which is associated with decreased proliferating chondrocytes and hypertrophic zone in these endochondral bones. This leads to premature loss of skull base sutures and smaller-than-normal long bones and vertebrae.⁵⁴ The expressions of chondrocyte markers *Ihh* and PTH-related peptide (*PTHrP*) are also diminished in *Fgfr2*^{IIIc}^{-/-} mice.⁵⁴ These results suggest that *Fgfr2*^{IIIc} regulates chondrocyte lineages. *Fgfr2*; *Dermol-Cre* mice have decreased bone length without apparent defects in chondrocyte proliferation, but show shortened hypertrophic zone, which is similar to that in *Fgfr2*^{IIIc}^{-/-} mice.⁵⁴ Increased osteoclast activity may account for decreased hypertrophic zone by increasing the removal of calcified hypertrophic chondrocyte matrix.³⁶

Genetic mouse models are also used to find therapeutic strategy. Activated ERK1/2 and p38 signaling pathways may participate in the regulation of coronal suture by FGFR2.^{39,49,52} The premature fusion of cranial sutures can be partially rescued by blocking ERK1/2 or p38 activation, respectively *in vitro* and *in vivo*.^{39,49,52,56} Shukla *et al.*^{56–57} showed that RNA interference targeting the mutant form of FGFR2 S252W completely rescued Apert-like syndrome in mice, and local treatment of the Apert mice with U0126,

an inhibitor of MEK1/2, significantly alleviated craniosynostosis. Using genetically modified mice simultaneously carrying C342Y, L424A and R424A mutations in the same FGFR2c (*Fgfr2*cCLR), researchers found that the activated FGFR2 signaling caused by C342Y mutation, cannot recruit and stimulate tyrosine phosphorylation of FRS2.⁵³ Thus, premature fusion of sutures, mediated by activated FGFR2IIIc, is prevented by attenuation of the signaling pathways through selective uncoupling between the docking protein FRS2 α and activated FGFR2IIIc.⁵³ These studies provide opportunities for developing novel therapeutic strategies for craniosynostoses.

FGFR3

FGFR3 is first expressed in chondrocytes, differentiated initially from the core of the mesenchyme condensation.⁵⁸ FGFR3 is expressed in reserve and proliferating chondrocytes as the epiphyseal growth plate is formed.^{12,58–59} Immunohistochemistry results have indicated that FGFR3 is also expressed in mature osteoblasts and in osteocytes.¹⁴ During calvarial bone development FGFR3 is expressed at low levels in sutural osteogenic fronts at the late stages (Table 1).^{34,38}

Gain-of-function point mutations in FGFR3 cause several types of the human skeletal dysplasias, including achondroplasia (ACH), hypochondroplasia (HCH), thanatophoric dysplasia (TD) and severe achondroplasia, with developmental delay and acanthosis nigricans (SADDAN).⁶⁰ Among these diseases, ACH is the most common type of human dwarfism characterized by short stature, especially in the proximal upper and lower limbs, central facial dysplasia, macrocephaly and spine protrusion.^{61–63} The phenotype of HCH is similar to ACH, but much milder than ACH, whereas TD is the most common form of lethal skeletal dysplasia characterized by macrocephaly, narrow bell-shaped thorax, severe shortening of the limbs and lethality in the neonatal period. TD has been classified into TDI and TDII. TDI patients have curved, short femurs, with or without cloverleaf skull, and TDII patients have relatively longer femurs with severe cloverleaf skull.⁶⁴ Patients with SADDAN exhibit acanthosis nigricans and anomalies in the central nervous system, in addition to severe skeletal dysplasia.^{65–66}

Currently, multiple FGFR3-related mouse models have been generated using genetic approach to study the role of FGFR3 in skeleton development and diseases. Mice carrying activating mutations of FGFR3 mimicking human ACH exhibit smaller body size, dome-shaped skull and shortened long bones with disorganized chondrocyte columns in growth plates.^{60,67–70} Mice carrying FGFR3 K644E mutation mimicking human TDII die within few hours after birth, whereas mice carrying FGFR3 S365C mutation, which corresponds to FGFR3 S371C mutation in human

TDI, exhibit skeletal dysplasia more severe than ACH.^{71–72} FGFR3 negatively regulates chondrogenesis of long bones by affecting the proliferative activity and differentiation of chondrocytes. A number of reports have demonstrated that FGFR3 signaling inhibits chondrocyte proliferation through STAT1 signaling by inducing the expression of cell cycle suppressor genes such as the CDK inhibitor p21.^{73–76} Loss of *Stat1* restored the reduced chondrocyte proliferation in ACH mice, but did not rescue the reduced hypertrophic zone or the delayed formation of secondary ossification centers in ACH mice. The expression of a constitutively active mutant of MEK1 in chondrocytes of *Fgfr3*-deficient mice inhibits skeletal overgrowth, strongly suggesting that FGFR3 inhibits chondrocyte differentiation through the ERK/MAPK pathway.⁷⁶ In contrast, evidence suggests that FGFR3 promote chondrocyte terminal hypertrophic differentiation.^{77–78} Conversely, mice carrying targeted deletion of FGFR3 exhibit overgrowth of long bone, wider hypertrophic zone, proliferative zone and enhanced proliferative activity of chondrocytes.^{59,79}

Moreover, the activity and the signaling outcomes of the FGFR3 pathway during chondrogenesis are also influenced by many intracellular and extracellular signals. Activated FGFR3 inhibits BMP4 expression in post-natal mouse growth plates,⁸⁰ while BMP treatment rescues the retarded growth of long bone in ACH mouse model.⁷⁷ These studies emphasize the antagonistic interaction between FGFR3 and BMP signaling in the control of chondrogenesis. Moreover, IHH expression is reduced in mice carrying activating FGFR3.⁸⁰ PTHrP partially reverses the inhibition of long bone growth caused by FGFR3 activation.⁷² It was suspected that FGFR3 signaling may act upstream of the IHH/PTHrP system in regulating the onset of hypertrophic differentiation.⁷⁷ In addition, it was reported that IGF1 prevents the apoptosis, induced by FGFR3 mutation, through the phosphoinositide 3-kinase pathway and MAPK pathways.⁸¹

FGFR3 signaling is also an important regulator of osteogenesis. Chondrocyte-specific activation of FGFR3 in mice causes premature synchondrosis closure and enhanced osteoblast differentiation around synchondroses. Premature synchondrosis closure is also observed in the spine and cranial base in human cases of homozygous ACH and TD, as well as in mouse models of ACH, with increased bone formation.^{70,72,82} Activated FGFR3 leads to decreased bone mass by regulating both osteoblast and osteoclast activities.^{83–84} Mice lacking FGFR3 also have decreased bone mineral density and osteopenia.^{14,85} FGFR3 can inhibit proliferation of BMSCs *in vitro*.^{83,85} However, both deletion and activation of FGFR3 can lead to increased differentiation, but impaired mineralization of osteoblasts (Figure 2).^{83,85} The reasons for these seemingly inconsistent results need to be explored.

Given its causal role in some skeletal disorders, including ACH, FGFR3 and/or its downstream pathways, are attractive targets for therapy. C-type natriuretic peptide is a newly identified potential therapeutic antagonist of FGFR3 signaling that alleviates the dwarfism phenotype of mice mimicking human ACH through its inhibition on FGFR3/MAPK pathway.^{86–87} It was reported that parathyroid hormone (PTH) (1–34) stimulates the longitudinal bone growth in rats and improves the growth of the cultured femurs from mice carrying a gain-of-function mutation (G380R) of FGFR3.^{88–89} In addition, we have found previously that PTHrP partially reversed the shortening of cultured bone rudiments from ACH mice.⁷² Recently, we found that systemic intermittent injection of PTH (1–34) can rescue the lethal phenotype of TDII mice and significantly alleviate the retarded skeleton development of ACH mice.⁹⁰ We also have identified a novel inhibitory peptide for FGFR3 signaling, which alleviated the bone growth retardation in bone rudiments from mice mimicking human TDII and reversed the neonatal lethality of TDII mice.⁹¹

FGFR4

In addition to its expression in the resting and proliferative zones of growth plates,¹¹ FGFR4 is also highly expressed in rudimentary membranous bone and strictly localized in osteoblasts between the periosteal and endosteal layers (Table 1).⁹² Interestingly, *Fgfr4*-deficient mice are developmentally normal, but the *Fgfr3/Fgfr4* double null mice grow more slowly.⁹³ However, the effect of FGFR4 on bone development remains unclear and needs further studies.

FGFS PARTICIPATE IN SKELETON DEVELOPMENT AND BONE METABOLISM

FGF2

FGF2 is one of the earliest members identified in the FGF polypeptide family, and is expressed in majority of cells and tissues including limb bud, chondrocytes and osteoblasts. FGF2 is stored in the extracellular matrix.^{11,94–96}

FGF2 contributes to the growth and patterning of the limb.⁹⁶ Overexpression of human FGF2 in mice (TgFGF2) results in dwarfism, with shortening and flattening of long bones and moderate macrocephaly.⁹⁷ Deletion of *Stat1* leads to a significant correction of the chondrodysplastic phenotype of TgFGF2 mice.⁹⁸ These results indicate the essential role of STAT1 in FGF-mediated regulation of epiphyseal growth plates. *Fgf2*-knockout (*Fgf2*^{−/−}) mice have normal limbs. The normal skeleton in *Fgf2*^{−/−} mice indicates that the function of FGF2 may be replaced by FGF8 and FGF4,⁹⁹ which is also expressed in the limb bud. FGF2 also plays important roles in bone homeostasis. Deletion of *Fgf2* in mice leads to decreased bone mass, bone formation and mineralization.^{95,100} Endogenous

FGF2 promotes the differentiation of bone marrow stromal cells (BMSCs) into osteoblasts, since FGF2 deficiency results in adipogenesis and reduced osteogenesis of BMSCs.^{95,101} Similar to *Fgf2*^{-/-} mice, TgFGF2 mice also have reduced bone mass, which may result from impaired endochondral ossification, or continuous exposure to high levels of FGF2 *in vivo*.^{14,102} Targeted overexpression of FGF2 in chondrocytes and osteoblasts should provide important information about the role of FGF2 in dwarfism and bone formation.¹⁰²

Other important factors for bone homeostasis also exert their effects through FGF2. PTH and BMP2-induced bone formation in *Fgf2*^{-/-} mice are greatly impaired, and osteoclast formation stimulated by PTH and BMP2 are also disrupted in *Fgf2*^{-/-} bone marrow stromal cultures.^{103–105} The impaired bone anabolic effect of PTH in *Fgf2*^{-/-} mice is associated with reduced expression of activating transcription factor 4, a critical regulator for osteoblast differentiation and function.¹⁰⁶ Furthermore, prostaglandin F2 α also induces osteoblast proliferation through endogenous FGF2.¹⁰⁷

FGF2 has three isoforms: a low molecular weight isoform (lmw, 18 kDa) and two high molecular weight isoforms (hwm, 21 and 22 kDa). FGF2lmw is secreted and activates FGFRs, whereas FGF2hwm remains intranuclear. Their roles in bone formation are largely unknown. Transgenic mice with targeted overexpression of FGF2lmw and FGF2hwm in immature and mature osteoblast lineage (via *Col3.6-cre*) are used to elucidate the differential functions of FGF2 isoforms in bone formation.^{108–109} *Col3.6-FGF2lmw* mice have increased bone mineral density (BMD), bone mass and enhanced mineralization of BMSCs, which is related to the reduced expression of the Wnt antagonist secreted frizzled receptor 1.¹¹⁰ In contrast to TgFGF2lmw mice, *Fgf2lmw*^{-/-} mice show significantly reduced BMD and impaired mineralization.¹⁰⁸

Col3.6-FGF2hwm mice display dwarfism, decreased BMD, increased FGF23 level, hypophosphatemia and rickets/osteomalacia, which is similar to X-linked hypophosphatemia (XLH).^{109–110} A potential mechanism is that FGF2 enhances FGF23/FGFR1/KLOTHO signaling, and then downregulates renal Na⁺/Pi cotransporter NPT2a, causing Pi wasting, osteomalacia and decreased BMD.¹⁰⁹ The upregulation of FGF23 level by FGF2hwm depends on FGFR1/MAPK pathway.¹¹⁰ These studies indicate that FGF2 isoforms have important effects on bone homeostasis and different FGF2 isoforms perform distinct roles.

FGF4

Vertebrate limb development largely depends on signals from the AER. During limb development, FGF4 is first expressed in the developing murine forelimb bud at E10.0. Its expression is strongest in the posterior AER at

E10.5–11.0 and is undetectable at E12.0.¹¹¹ FGF4 provides mitogenic and morphogenic signals to regulate normal limb development.^{111–112} *Fgf4* knockout (*Fgf4*^{-/-}) mice die on E4.5 (early embryonic stages),¹¹³ preventing the direct evaluation of FGF4 function in the developing limb. Mice with targeted deletion of *Fgf4* in limbs (via *Rarb-Cre*) are viable and have normal skeletal patterns.¹¹¹ The expression pattern of Sonic hedgehog (Shh), another key signaling molecule in AER maintenance, is normal in the limb buds, suggesting that FGF4–Shh feedback loop is not essential for limb development.

In addition to its essential roles in the AER of normal embryo, FGF4 can also promote intramembranous ossification and participate in the development of calvarial bone. FGF4 is expressed in sutural mesenchyme during early craniofacial skeletogenesis.⁶⁰ Treatment with FGF4 on developing mouse coronal suture leads to synostotic coronal sutures accompanied by the induction of apoptosis and accelerated mineralization.¹¹⁴ FGF4 can also cause premature suture fusion with increased cell proliferation, both in cultured calvaria and in mice.¹¹⁵ Furthermore, systemic administration of FGF4 and its 134 amino-acid residues leads to increased bone formation in rats and mice *in vivo*.¹¹⁶ FGF4 can also promote BMSC proliferation *in vitro*,^{117–118} and strongly stimulate Runx2 expression in osteoblast-like MC3T3-E1 and murine premyoblast C2C12 cells.¹¹⁹ However, studies especially genetic studies on the role of FGF4 in bone formation, are still lacking.

FGF8

FGF8 is expressed throughout the AER, indicating its important role in limb development.^{120–122} Mice with deleted *Fgf8* show early embryonic lethality before limb development.^{123–124} Lewandoski *et al.* generated mice with targeted deletion of *Fgf8*¹²⁴ (via *Mx2-cre*) in limb bud.¹¹² These mice display failed limb development with substantial reduction in limb-bud size, and hypoplasia or aplasia of specific skeletal elements.¹¹² However, the *Mx2* promoter driven cre is not expressed sufficiently early to completely ablate *Fgf8* function during forelimb formation, which results in a complex forelimb phenotype. Using *Rarb-Cre* mice, *Fgf8* is conditionally deleted in the developing forelimb AER. These mice have severe forelimb deformity, including the absence of radius and first digit.^{125–126}

In addition to its important role in limb development, FGF8 also regulates osteoblast and chondrocyte differentiation. FGF8 is expressed in chondrocytes and perichondrium of dorsal costal bone, as well as in the osteoblast compartment of calvarial bone in cortical bone and the growth plate of developing bones.^{60,127} FGF8 can effectively predetermine mouse BMSCs and C2C12 cell line to differentiate to osteoblasts and increase bone formation *in vitro*.^{128–129} However, Lin *et al.*¹³⁰ found

that FGF8 stimulated the proliferation of MC3T3E1 or primary rat osteogenic cells, but inhibited osteogenic differentiation and mineralization. These controversial results may be attributed to the different cells used in *in vitro* experiments. As to cartilage, FGF8 can promote the degradation of cartilage and exacerbation of osteoarthritis.¹³¹ However, the influence of FGF8 on bone and cartilage remains unclear.

FGF9

FGF9 has the highest affinity to FGFR3, and can also bind FGFR2 with a lower affinity (Table 1).¹³² FGF9 is broadly expressed in different tissues including in AER, perichondrium/periosteum, chondrocytes of growth plate, as well as primary spongiosa.^{133–135}

Colvin *et al.*¹³⁶ generated *Fgf9* knockout (*Fgf9*^{−/−}) mice and showed that deletion of *Fgf9* alleles led to lethality at the neonatal stage mainly due to malformations of the lung, and causing male-to-female sex reversal.^{136–137} *Fgf9*^{−/−} mice display disproportionate shortening of the proximal skeletal elements (rhizomelia), but the limb bud development and mesenchymal condensations are normal.¹³⁵ These results indicate that loss of *Fgf9* in AER does not lead to limb patterning defects that primarily affected mesenchymal condensation. The rhizomelia results from the loss of *Fgf9* function after mesenchymal condensation. Similarly, transgenic mice, with overexpression of *Fgf9* in chondrocytes (*Col2a1-Fgf9*), also show dwarfism, short limb and vertebral defect because of the reduced proliferation and terminal differentiation of chondrocytes. These results are similar to bone phenotypes, caused by activated FGFR3.¹³³ These seemingly inconsistent results between *Fgf9* null and transgenic mice may result from distinct effect of FGF9 on different stages of skeletogenesis.

In addition, *Fgf9*^{−/−} mice also show impaired osteogenesis, which may be secondary to the earlier defective chondrogenesis and vascularization,¹³⁵ or FGF9 may directly regulate osteogenesis, as demonstrated by *in vitro* calvarial bone cell culture studies.¹³⁸ Furthermore, the loss of *Fgf9* results in a deficiency of osteoclasts in the perichondrium and primary spongiosa of developing bone.¹³⁵ These findings suggest that FGF9 can positively regulate osteogenesis and osteoclastogenesis in endochondral ossification.

FGF9 is also expressed in the mesenchyme of suture in the early craniofacial development stages.¹¹⁵ By contrast to its promoting effects on osteogenesis in endochondral ossification, targeted overexpression of FGF9 in cranial mesenchymal cells leads to a switch from intramembranous to endochondral ossification in mouse parietal bones, indicating that FGF9 may regulate bone development by affecting the direction of mesenchyme differentiation.¹³⁹

Recently, missense mutations in FGF9 have been identified to result in elbow-knee synostosis, premature fusion of cranial sutures in mice¹⁴⁰ and multiple synostosis syndrome in humans.¹⁴¹ These data further suggest the important effect of FGF9 on bone development.

However, the different impacts of FGF9 on different stages of limb development and the direct effect of FGF9 on adult bone homeostasis are still unclear. Targeted deletion of *Fgf9* in different stages and cells using *Fgf9* CKO mice¹⁴² are necessary to answer these questions in the future.

FGF10

FGF10 is expressed in the lateral plate mesoderm and serves as a mesenchymally expressed limb bud initiator,^{44,143–144} and the expression persists in the mesenchyme under AER after initial limb bud formation. FGF10 acts epistatically at the upstream of FGF8.¹⁴⁵ Positive feedback exists between FGF8 and FGF10, which is essential for limb development.⁴⁴ To define the role of FGF10, *Fgf10* knockout (*Fgf10*^{−/−}) mouse strain was generated. These mice show complete absence of fore- and hindlimbs, and die after birth associated with complete absence of lungs.^{145–146} The limb bud formation in *Fgf10*^{−/−} embryos is initiated but outgrowth of the limb buds is impaired, while the clavicle formation is normal.¹⁴⁶ However, the impact of FGF10 on postnatal bone development and modeling remains unclear.

FGF18

FGF18 is expressed in osteogenic mesenchymal cells and differentiating osteoblasts of developing calvaria, in the perichondrium and joints, as well as growth plates of developing long bones.^{11,147–148}

Fgf18 knockout (*Fgf18*^{−/−}) mice die shortly after birth, and display expanded zones of proliferating and hypertrophic chondrocytes with increased chondrocyte proliferation and differentiation, similar to that observed in mice lacking *Fgfr3*.^{147–148} Bone cultures of fetal mouse tibias treated with FGF18 show decreased bone length and hypertrophic differentiation of chondrocytes.^{87,149} These studies demonstrate the inhibitory effect of FGF18 in chondrogenesis. In contrast to the negative role of FGF18 in chondrogenesis found in *Fgf18*^{−/−} mice or FGF18-treated cultured bone, the proliferation and differentiation of primary chondrocytes and prechondrocytic ATDC5 cells are stimulated by FGF18 treatment *in vitro*.¹⁵⁰ FGF18 also enhances BMP function and stimulate chondrogenesis in earlier stages of cartilage formation by suppressing noggin expression.¹⁵¹ These seemingly contradictory data suggest that the *in vivo* role of FGF18 in chondrogenesis need to be further studied. In addition, FGF18 regulates bone development by inducing skeletal vascularization and

subsequent recruitment and formation of osteoclasts in developing long bone.¹⁵²

Fgf18^{-/-} mice also show delayed suture closure with decreased proliferation of calvarial osteogenic mesenchymal cells and delayed osteogenic differentiation. The calvarial bone mineralization in *Fgf18*^{-/-} mice is also decreased.^{148,152} The delayed osteogenic differentiation is also observed in the developing long bones of *Fgf18*^{-/-} mice.¹⁵² *In vitro* studies show that FGF18 treatment results in enhanced proliferation of MC3T3-E1 cells and perichondrial cells in cultured metatarsals,¹⁵⁰ supporting the promoting effect of FGF18 on osteogenesis. These data indicate that FGF18 may be an important modulator for both endochondral and intramembranous bone formation in adult mice.

Although FGF18 is a key regulator for chondrogenesis, osteogenesis and vascularization of early skeleton development, the mechanism and the direct effect of FGF18 on the three critical stages in skeleton developmental or bone homeostasis at adult period need to be further studied.

FGF21

FGF21 is a member of the FGF19/21/23 subfamily that functions as an endocrine hormone.^{153–154} FGF21 is a powerful regulator of glucose and lipid metabolism.^{155–158} Recently, FGF21 has also been found to participate in bone homeostasis. The overexpression of *Fgf21* in liver driven by ApoE promoter in transgenic mice show decreased bone mass, impaired bone formation and increased osteoclast function, which is consistent with the phenotypes of mice with pharmacological FGF21 treatment. In contrast, *Fgf21*^{-/-} mice have increased bone mass with improved osteogenesis and decreased osteoclast function. The possible mechanism is that FGF21 stimulates adipogenesis from bone marrow mesenchymal stem cells by potentiating the activity of peroxisome proliferator-activated receptor γ , but inhibits osteoblastogenesis.¹⁵⁹ These results indicate that FGF21 is a negative regulator of bone turnover and a key integrator of bone and energy metabolism, and underscores the importance of the whole body energy metabolism in bone physiology.¹⁵⁹

Furthermore, FGF21 is expressed in the growth plate,^{160–161} and is associated with reduced skeletal growth and growth hormone (GH) insensitivity caused by undernutrition. After food restriction, FGF21 expression is increased in the tibial growth plates of mice. *Fgf21*^{-/-} mice exhibit greater body and tibia growth than their wild-type controls after food restriction because of reduced GH binding and GH receptor expression in the liver and in the growth plates of wild-type mice, but not in that of *Fgf21*^{-/-} mice.¹⁶¹ FGF21 also has direct effect on chondrocytes. Higher concentrations of FGF21 inhibit chondrocyte proliferation and differentiation by reducing

GH binding in cultured chondrocytes.¹⁶⁰ FGFR1 may participate as receptors of FGF21 in the regulation of chondrocytes by FGF21.^{160,162}

Owen *et al.*¹⁶³ found that physiological levels of FGF21 regulate the HPA axis and glucocorticoid levels, as well as the kisspeptin pathway in female fertility, which may also have effect on bone homeostasis.

FGF23

FGF23 is an approximately 32-kDa protein with an N-terminal FGF homology domain and a novel 72-amino-acid C-terminus, which permits interaction with FGF receptor- α -Klotho coreceptor complexes in cell membranes of target tissues.^{31,164} FGF23 is mainly secreted by osteoblasts and osteocytes,^{165–167} and as a hormone to regulate systemic phosphate homeostasis and vitamin D metabolism.

FGF23 downregulates serum phosphate. Mutations in an RXXR site in FGF23 prevents its cleavage resulting in autosomal-dominant hypophosphatemic rickets (ADHR), characterized by low serum phosphorus concentrations, rickets, osteomalacia, lower extremity deformities, short stature, bone pain and dental abscesses.^{168–172} The overproduction of FGF23 by tumors¹⁷³ and osteogenic cells in fibrous dysplastic lesions¹⁷⁴ may be responsible for the hypophosphatemia in tumor-induced osteomalacia and fibrous dysplasia, respectively. In addition to its role in hypophosphatemic diseases, FGF23 is involved in hyperphosphatemic diseases. Hyperphosphatemic familial tumoral calcinosis is a relatively rare genetic disease characterized by enhanced renal tubular phosphate reabsorption and elevated serum phosphorus, as well as paraarticular calcific tumors.¹⁷⁵ Multiple mutations in FGF23 gene that lead to decreased FGF23 activity have been identified in patients with hyperphosphatemic familial tumoral calcinosis.^{176–178} These human studies help to define the critical role of FGF23 in regulating phosphate metabolism.

The transgenic mice, ubiquitously expressing human FGF23, reproduce the common clinical features of hypophosphatemia, including decreased serum phosphorus concentration, increased renal phosphate wasting, inappropriately low serum 1,25-dihydroxyvitamin D [1,25(OH)₂D] level, and rachitic bone.¹⁷⁹ Overexpression of human FGF23 in osteoblastic lineage or FGF23R176Q (a mutant form that fails to be degraded by furin proteases) in liver results in phenotypic changes similar to those of patients with ADHR or transgenic mice expressing FGF23 ubiquitously.^{180–181} Serum phosphate level is regulated by renal NaPi-2a in the brush border membrane of proximal tubules.¹⁸² The renal phosphate wasting in the transgenic mice is accompanied by the reduced expression of NaPi-2a.¹⁷⁹ The reduction of serum 1,25(OH)₂D levels may result

from a significant decrease in renal mRNA level for 25-hydroxyvitamin D-1 α -hydroxylase (1 α -OHase) and a simultaneous elevation of 24-hydroxylase mRNA, induced by increased serum level of FGF23 (Figure 3).¹⁸³

Consistently, *Fgf23* knockout (*Fgf23*^{-/-}) mice have opposite features including significantly increased serum levels of phosphate, calcium and 1,25(OH)₂D because of the upregulated renal phosphate reabsorption and enhanced expression of renal 1 α -OHase, respectively.¹⁸⁴ The *Fgf23*^{-/-} mice also exhibit premature aging-like phenotypes including reduced lifespan, infertility, osteoporosis and renal dysfunction.¹⁸⁴ The elimination or reduction of vitamin D activity from *Fgf23*^{-/-} mice can rescue the premature aging-like features and ectopic calcifications. These *in vivo* experimental data strongly support the very essential roles of FGF23 in the regulation of phosphate homeostasis, vitamin D activity and in the pathogenesis of premature aging.¹⁸⁵

Recent studies have indicated the regulation of iron on FGF23. Reduced serum iron concentrations are strongly correlated with increased serum FGF23 in ADHR patients,¹⁸⁶ and C-terminal FGF23 is negatively correlated with ferritin.¹⁸⁷ To investigate the effect of iron on the development of the ADHR phenotype, R176Q-*Fgf23* knock-in mice mimicking human ADHR are generated and placed on control or low-iron diets.^{188–189} R176Q-*Fgf23* knock-in mice on low-iron diet have elevated intact C-terminal *Fgf23* with hypophosphatemic osteomalacia and low serum 1,25(OH)₂D. Iron chelation *in vitro* results in a significantly increased *Fgf23* mRNA level that depends on MAPK signaling.¹⁸⁹ However, the mechanism for the regulation of FGF23 by iron is still unclear.

Increased FGF23 level is also found in patients with hypophosphatemic diseases including XLH and autosomal

dominant hypophosphatemic rickets (ARHR). XLH is caused by inactivating mutations in phosphate regulating gene with homologies to endopeptidases on the X chromosome (PHEX).^{190–191} Mice with ablation of *Phex* gene (Hyp mice) have increased FGF23 expression and hypophosphatemia.¹⁹² Both the serum phosphate levels and skeletal changes in Hyp mice can be reversed by introducing *Fgf23* null mutation into Hyp mice,^{166,193–194} indicating that enhanced FGF23 level is responsible for the hypophosphatemia in XLH patients and Hyp mice. The increased FGF23 level is due to the improved *Fgf23* expression, but not decreased degradation.^{165,194–195} ARHR results from missense mutations in DMP-1. *Dmp1* knockout mice exhibit hypophosphatemic rickets and osteomalacia similar to ARHR patients.^{196–197} Both *Dmp1* null mice and patients with ARHR show elevated serum FGF23 levels. Considering the role of FGF23 in ADHR and other hypophosphatemic diseases, ARHR has been proposed to be associated with excessive actions of FGF23.

FGF23 also participates in some clinical pathological processes, in addition to its role in genetic diseases. In patients with chronic kidney disease (CKD), FGF23 level is elevated due to increased serum calcium and phosphate concentrations and PTH,^{31,198} and is associated with increased FGF23 transcription in bone.¹⁹⁹ Some researchers proposed that FGF23 might be an early biomarker for earlier interventions in CKD.²⁰⁰ However, the reason for the high serum levels of FGF23 in CKD is unclear. Furthermore, elevated level of FGF23 in CKD patients have been linked to greater risks of left ventricular hypertrophy (LVH).^{201–202} Using animal models, Faul *et al.*²⁰³ found that increased level of FGF23 in mice resulted in pathological hypertrophy of cardiomyocytes and LVH. To avoid redundancy and

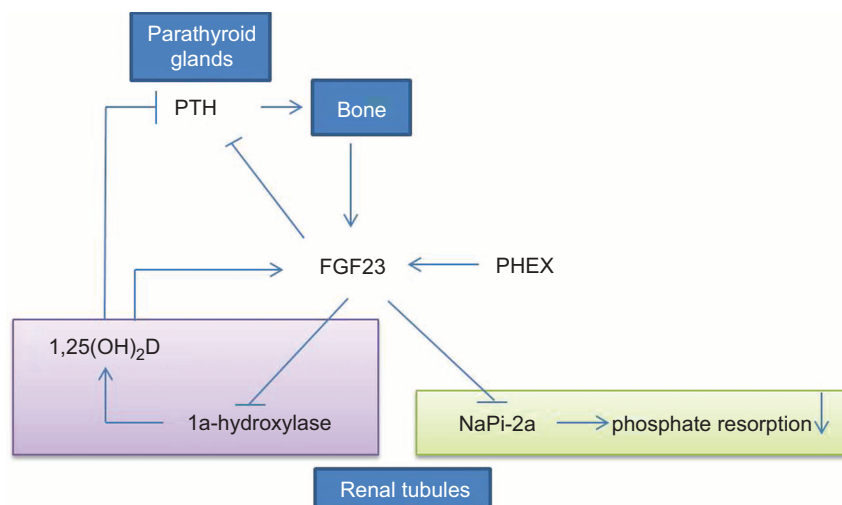


Figure 3. FGF23 regulates systemic phosphate homeostasis and vitamin D metabolism. FGF23 can reduce expression of NaPi-2a in kidney tubules and lead to renal phosphate wasting. FGF23 downregulates activity of 25-hydroxyvitamin D 1 α -hydroxylase in kidney tubules and reduces 1,25(OH)₂D level. Furthermore, FGF23 also have relationship with PTH and PHEX.

Table 2 Mouse models with genetically modified FGF/FGFR signaling in skeletal development and homeostasis

Gene	Model	Exon	Cre line (tissue)	Survival	Phenotype	Reference	Related human skeleton disease
FGFR1	KO	Exon 4	Germline	Die at E7.5-9.5	Severe growth retardation, defect of mesodermal differentiation	[17]	NA
	KO	Exons 8-14	Germline	Die at E7.5-9.5	Early growth defects, aberrant mesodermal patterning	[18]	NA
	FGFR1-deficient ES chimeras	Exon 4; Exons 8-14	Germline	Die during gastrulation	Defective cell migration through primitive streak, malformation of chimeric limb buds	[21,228]	NA
	KO	Exon 3 (α -isoforms)	Germline	Die at E9.5-12.5	Distal truncation of limb bud, lethal at E9.5-12.5 due to posterior embryonic defects	[20]	NA
	KO	Exon 8 (IIIb)	Germline	Viable	No obvious phenotype	[19]	NA
	KO	Exon 9 (IIc)	Germline	Lethal	Gastrulation defects	[19]	NA
	KO	Exons 8-17 (Frs2/3-binding site)	Germline	Die during late embryogenesis	Defects in neural tube closure and in the development of the tail bud and pharyngeal arches	[25]	NA
	CKO	Exons 8-14	T (brachyury)-cre (all LMB cells)	Die at birth	Later reduction of limb skeleton, misshapen forelimb/hindlimb bud, missing digits	[10,229]	NA
			<i>Shh-cre</i> (posterior LBM cells)		Normal limb bud size, missing digit 3	[10,229]	NA
			<i>Ap2-Cre</i> (progress zone of the mouse limb at E10.5)		Abnormal development of the anterior digits	[24]	NA
			<i>Hoxb6-Cre</i> (lateral plate mesoderm of E8.5)		Severe abnormalities in autopod formation in hindlimbs	[24]	NA
	CKO	Exons 8-15	<i>Col2a1-cre</i> (osteochondrocyte lineage)	Viable	Increased bone mass, delayed osteoblast differentiation, increased proliferation of osteochondro-progenitor cells, increased height of the hypertrophic chondrocyte zone at E16.5	[15,230]	NA
			<i>Col1-cre</i> (differentiated osteoblasts)	Viable	Increased bone mass, accelerated osteoblast differentiation and mineralization, impaired osteoclast activity	[15,230]	NA
			<i>LysM-cre</i>	Viable	Increased bone mass, impaired osteoclast formation and activity	[29]	NA
			<i>OC-cre</i>	Viable	Increased bone mass	Su <i>et al.</i> unpublished data	NA
	CKO	Exon 4	<i>Meox2-Cre</i> (various)	Die at E11.5	Developmental delay, mesodermal migration and patterning defects, craniorachischisis and posterior truncations	[25]	NA
	DN	Transgene (Tyrp1-FGFR1*IIc)	Retinal pigment epithelium		No skeleton phenotype was reported	[231]	NA
	GOF (KI)	Exon 7 (P250R)	Germline	Viable	PS including decreased body size, premature suture closure, increased bone formation at suture	[28]	PS (P250R)
	OE	Transgene (BAC-FGFR1P252R)	Various	Viable	Premature suture closure	[232]	PS (P250R)

Continued

Table 2. Continued

Gene	Model	Exon	Cre line (tissue)	Survival	Phenotype	Reference	Related human skeleton disease
FGFR2	KO	Exons 10,11 and part of exon 12 (transmembrane domain and part of its kinase I domain)	Germline	Die at E4.5-5.5	The growth of the inner cell mass stopped, no visceral endoderm formed, trophoblast defects	[43]	NA
	KO	Exons 7-9 (Entire Ig III)	Germline	Die at E10-11	Failure of limb bud initiation and placenta formation	[44,45]	NA
	KO-LacZ	Exon 8 (IIlb)	Germline	Die at birth	Impaired limb outgrowth, severe dysgenesis of multiple organs	[233]	NA
	KO	Exon 9 (IIlc) (A translational stop codon inserted into exon 9)	Germline	Viable	Delayed ossification in the sphenoid region of the skull base, dwarfism in the long bones and axial skeleton	[54]	NA
	CKO	Exon 8 (IIlb)	<i>CMV-Cre</i> (germline)	Die at birth	Defects of limb outgrowth and branching morphogenesis	[234]	NA
	CKO	Exon 9 (IIlc) (Resulting in a GOF mutation associated with exon switching within the <i>Fgfr2</i> gene)	<i>ZP3-Cre</i> (germline)	Die within 9 days	Coronal synostosis, ocular proptosis, precocious sternal fusion, and abnormalities in secondary branching in several organs	[235]	CS/PS
	CKO	Exons 8-10	<i>Dermo1-Cre</i> (mesenchymal condensations)	Viable	Skeletal dwarfism and decreased bone density, impaired proliferation of osteoprogenitors and function of mature osteoblasts	[36]	NA
	KD (RNAi)	Transgene (U6-ploxPneo- <i>Fgfr2</i>)	<i>El/la-Cre</i> (germline)	Lethal	Displayed limb defects	[57]	NA
	GOF (KI)	Exon 7 (S250W)	Germline	Viable	Several features similar to AS including smaller body size, brachycephaly, and midface hypoplasia	[51]	AS
	GOF (KI)	Exon 7 (S252W)	Germline	Neonatal lethality	Smaller size, midline sutural defect and craniosynostoses, increased cartilage in the basicranium, nasal turbinates and long bone	[48]	AS(S252W)
	GOF (KI)	Exon 7 (P253R)	Germline	Viable	Smaller body size, brachycephaly and syndactyly, premature of cranial sutures	[39]	AS (P253R)
	GOF (KI)	Exon 7 (P253R)	Germline	Die at P1-3w	Smaller body size, brachycephaly and syndactyly, premature of cranial sutures	[49]	AS (P253R)
	GOF (ENU-induced)	Exon 7 (W290R)	Germline	Neonatal lethality	Features resembling those found in patients with CS	[236]	CS
	GOF (KI)	Exon 9 (IIlc) (C342Y)	Germline	Viable	Shortened face, protruding eyes, premature fusion of cranial sutures, and enhanced Spp1 expression in the calvaria, just like human Crouzon syndrome/Pfeiffer syndrome	[55]	CS/PS (C342Y)
	GOF (KI)	Exon 9 (IIlc) and Exon 10 (transmembrane domain) (C342Y; L424A; R424A, CLR)	Germline	Viable	Normal skull development	[53]	NA
	GOF (KI)	Exon 10 (transmembrane domain) (Y394C)	Germline	Postnatal lethality	Epidermal hyperplasia and premature closure of cranial sutures (craniosynostosis) due to abnormal cell proliferation and differentiation	[52]	BSS

Continued

Table 2. Continued

Gene	Model	Exon	Cre line (tissue)	Survival	Phenotype	Reference	Related human skeleton disease
FGFR3	KO	Exon 5	Germline	Viable	Bone overgrowth, decreased bone mass	[79]	CATSHL syndrome
	KO	From Ig-like domain II to the transmembrane domain	Germline	Viable	Bone overgrowth, defective bone mineralization and osteopenia, early arthritis, deafness	[59,85,237]	CATSHL syndrome
	KO (a stop codon inserted)	Exon 8 (IIb)	Germline	Viable	No obvious phenotype	[238]	NA
	KO (a stop codon inserted)	Exon 9 (IIc)	Germline	Viable	Skeletal overgrowth, decreased bone mineral density	[238]	NA
	CKO	Exons 9–10	<i>E1/a-Cre</i>	Viable	Increased length of long bone and decreased bone mineral density	[239]	NA
	GOF (KI)	Exon 7 (P244R)	Germline	Viable	Abnormal craniofacial morphology	[240]	MS (P250R)
	GOF (KI)	Exon 9 (Y367C)	Germline	Viable (die at 6–8 weeks after birth)	Skeletal dysplasia more severe than ACH	[241]	TD I (Y373C)
	GOF (KI)	Exon 10 (S365C)	Germline	Viable	Skeletal dysplasia more severe than ACH	[72]	TD I
	GOF (KI)	Exon 10 (G369C)	Germline	Viable	Macrocephaly and shortened limbs due to retarded endochondral bone growth and premature closure of cranial base synchondroses	[70]	ACH (G375R)
	GOF (KI)	Exon 10 (G374R)	Germline	Viable	Small size, short tail, macrocephaly and dome-shaped heads, the narrower epiphyseal growth plates and decreased hypertrophic chondrocyte zone	[67,68]	ACH (G380R)
	GOF (KI)	K644E cDNA knock-in	Germline	Viable	Retardation of bone growth, macrocephaly and shortening of the long bones resembling ACH patients	[73]	ACH
	GOF (KI)	Exon 15 (K644E)	Germline	Neonatal lethality	Die within few hours after birth, skeletal dysplasia more severe than ACH	[71]	TD II (K650E)
	GOF (KI)	Exon15 (K644M)	Germline	Viable	Acanthosis nigricans and anomalies in central nervous system in addition to severe skeletal dysplasia	[242]	SADDAN
	OE	Transgene (Col2- G374R)	Chondrocyte	Viable	Mice are dwarfed, with axial, appendicular and craniofacial, skeletal hypoplasia	[80]	ACH
	OE	Transgene (FGFR3-hG380R)	Germline	Viable	Disproportionate dwarfism similar to those of human achondroplasia	[243]	ACH
FGFR4	KO	Exon 6 (Ig II)	Germline	Viable	Morphologically normal, no obvious defects in skeleton	[93]	NA
	KI	Exon 8 (G385R)	Germline	Viable	Skeleton phenotype not reported	[244]	NA
FGFR3/FGFR4	Double KO		Germline	Viable	Neonatal growth retardation, lung abnormalities	[93]	NA
FGF1	KO	Exon1	Germline	Viable	No obvious phenotype	[206]	NA

Continued

Table 2. Continued

Gene	Model	Exon	Cre line (tissue)	Survival	Phenotype	Reference	Related human skeleton disease
FGF2	KO	Exon1 (all three isoforms)	Germline	Viable	Impaired cerebral cortex development, blood pressure regulation	[245]	NA
	KO	Exon1 (all three isoforms)	Germline	Viable	Decreased bone mass, decreased vascular smooth muscle contractility, low blood pressure and thrombocytosis	[95,100]	NA
	KO	Exon1 (All three isoforms)	Germline	Viable	Delayed wound healing and neuronal defects and impaired development of the cerebral cortex	[99]	NA
	KO	Exon 1 (CTGCAG replacing the wild-type CCATGC) (Lmw)	Germline	Viable	Decreased bone mineral content, bone, BMD and impaired mineralization of BMSCs	[108,246]	NA
	KO	Exon 1 (the 14-bp oligo was designed to introduce stop codons in all three reading frames) (hmw)	Germline	Viable	Skeleton phenotype was not reported	[247]	NA
Heterozygous (<i>Fgf2</i> ^{+/-})	OE	Exon1	Germline	Viable	Decreased bone mass and bone formation	[104]	NA
	OE	Transgene (PGK-hFGF2)	Various	Viable	Dwarf mouse with premature closure of the growth plate and shortening of bone length, defective bone mineralization and osteopenia	[97,102]	NA
	OE	Transgene (3.6 kb) Col1a-18-kDa FGF2-IRES-GFPsaph	Immature and mature osteoblast lineage	Viable	Increased BMD, bone volume, trabecular thickness, and cortical bone thickness	[108]	NA
	OE	Transgene (3.6 kb) Col1a-HMW	Immature and mature osteoblast lineage	Viable	Dwarfism, decreased BMD, osteomalacia, increased FGF23 level and hypophosphatemia	[109,110]	similar to XLH
FGF3	KO	Exon1b (leaky expression of the mutant <i>Fgf3</i>)	Germline	Die in the early postnatal period	A short, dorsally curled tai and caudal vertebrae, smaller body, inner ear defects	[205]	NA
	CKO	Exons1b-3	<i>El/la-Cre</i> (germline)	Viable	Shortened, thickened and curved tail, normal inner ears	[248,249]	NA
	CKO	Exon 2	<i>CMV-cre</i> (germline)	Viable	Short, curly tails, abnormal otic morphologies	[250]	NA
	CKO	Exon 2		Viable	Not used for skeleton research	[251]	NA
FGF3/4	OE	Upregulation of FGF3/4 caused by retroviral insertion	Cranial sutures	Viable	Facial shortening with increased interorbital distance and precocious closure of several cranial sutures (craniosynostosis)	[252]	Craniosynostosis
FGF4	KO	Exon 1	Disrupted prior to limb bud initiation	Died at E5.0	Severely impaired proliferation of the inner cell mass	[113]	NA
	CKO-AP	Exons 1-3	<i>Rarb/Cre</i> (developing forelimb region)	Viable	Normal forelimbs and hindlimbs	[111,126]	NA
	CKO	Exons 2-3	<i>MSX2-cre</i>	Viable	Normal forelimbs and hindlimbs	[112]	NA
FGF5	KO	Exon 1	Germline	Viable	Abnormally long hair, impaired skeletal muscle	[207]	NA
FGF6	KO-Lac Z	Exon 1	Germline	Viable	No abnormal phenotype of skeleton detected	[208]	NA
FGF7	KO	Exon 1	Germline	Viable	No abnormal phenotype of skeleton detected	[209]	NA

Continued

Table 2. Continued

Gene	Model	Exon	Cre line (tissue)	Survival	Phenotype	Reference	Related human skeleton disease
FGF8	KO	Exons 2–3+ neo	Germline	Lethal	Early embryonic lethality before limb development	[123,124]	NA
	CKO	Exons 2–3	<i>β-actin-cre</i> (early embryo)	Lethal	Early embryonic lethality	[123,124]	NA
			<i>MSX2-cre</i> (functions initiated after FGF8 expression in forelimb, but before FGF8 expression in hindlimb)	Not mentioned	Substantial reduction in limb-bud size, and hypoplasia or aplasia of specific skeletal elements	[112]	NA
FGF9	CKO-AP	Exon 5	<i>Lefty2-Cre</i> (mesoderm)	Not mentioned	Limb bud development proceeded normally	[125,126]	NA
			<i>Rarb-Cre</i> (developing forelimb region)	Not mentioned	Severe forelimb deformity including absence of radius and first digit	[125,126]	NA
			<i>AP2-Cre</i> (limb bud ectoderm of E9.5 embryos)	Not mentioned	Absence of both forelimbs and hindlimbs	[126]	NA
FGF10	KO	Exon 1	Germline	Die at birth	Lung hypoplasia, male-to-female sex reversal, inner ear morphogenesis defect, slightly smaller body, short proximal skeletal	[135–137,253]	NA
	Heterozygous (<i>Fgf9</i> ^{+/−})	Exon 1	Germline	Viable	Reduced bone regeneration, impaired neovascularization and decreased cell proliferation	[254]	NA
	CKO	Exon 1	<i>Nestin Cre</i> (germline)	Die at birth	Lung hypoplasia, skeleton phenotype not mentioned	[142]	NA
FGF11	OE	Transgene (α A-crystallin-FGF9)	Cranial mesenchymal cells	Viable	Parietal bones show a switch from intramembranous to endochondral ossification	[139]	NA
		Transgene (Col2a1-FGF9)	Chondrocyte	Viable	Short limb, vertebral defect, reduced proliferation and terminal differentiation of chondrocytes	[133]	NA
	GOF (Spontaneous mutation)	N143T	Germline	Lethal	EKS with radiohumeral and tibiofemoral synostosis, craniostosis; lung hypoplasia	[140,255]	EKS (158)
FGF12	LOF (ENU screen)	Y162C	Germline	Viable	Normal skeleton phenotype, no male-to-female sexual reversal, decreased vision and retarded lens growth	[256]	NA
		Exon 1	Germline	Perinatal lethality	Complete absence of both fore- and hindlimbs, pulmonary branching morphogenesis was completely disrupted	[145]	NA
		Exon encoding the ATG translational start site	Germline	Die after birth	Complete truncation of the fore- and hindlimbs, normal clavicles, lung defect	[146]	NA
FGF13	CKO	Exon 2	Germline	Viable	Skeleton phenotype was not analyzed	[251]	NA
		Insertion of Velocigene cassette ZEN-Ub1	Germline	Viable	Skeleton phenotype was not analyzed	[219]	NA
		Exon 2	Germline	Viable	Skeleton phenotype was not analyzed	[213]	NA
FGF14	CKO	Exons 2–3	Germline	Viable	Skeleton phenotype was not analyzed	[214]	NA
		Exon 2	Germline	viable	Skeleton phenotype was not analyzed, developed ataxia and a paroxysmal hyperkinetic movement disorder; reduced responses to dopamine agonists	[215]	NA
					Skeleton phenotype was not analyzed, enhanced bile acid synthesis and contracted gallbladder	[216]	NA
FGF15	KO	Exon 3	Germline	Die at E13.5–P21	No bone phenotype was analyzed, decreased proliferation of embryonic cardiomyocytes	[217]	NA
FGF16	KO-LacZ	Exons 2–3	Germline	Viable	Normal skeletal patterns, abnormal cerebellar development and social behaviors	[210,211]	NA
FGF17	KO	Exons 1a–1b	Germline	Viable			

Continued

Table 2. Continued

Gene	Model	Exon	Cre line (tissue)	Survival	Phenotype	Reference	Related human skeleton disease
FGF18	KO	Exon 3	Germline	Die just before or at birth	Impaired ossification and increased chondrocyte proliferation, decreased alveolar spaces in the lung	[148,257]	NA
	KO-LacZ	Exon 1	Germline	Die after birth	Impaired ossification and increased chondrocyte proliferation, respiratory failure	[147,152]	NA
FGF20	Heterozygous	Exon 3	Germline	Viable	Reduced bone regeneration	[258]	NA
	CKO	Exon1	Germline	Viable	Even not be used in bone development	[259]	NA
	KO-LacZ	Exon1	Germline	Viable	No bone phenotype was analyzed, deafness	[218]	NA
	KO	Exons 2, part of exon1 and exon3	Germline	Viable	Greater body and tibia growth after food restriction	[157,161]	NA
	KO (LacZ)	Exons1-3	Germline	Viable	Skeleton phenotype was not reported	[156]	NA
FGF21	CKO	Exons1-3	<i>Meox-cre</i> (Germline)	Viable	Increased bone mass, metabolic defects including decreased circulating glucose level and oxygen consumption	[155,159]	NA
	OE	Transgene (Apoe-FGF21)	Liver	Viable	Decreased bone mass, increased osteoblast and bone resorption	[158,159]	NA
	OE	Transgene (Apoe-hFGF21)	Liver	Viable	Skeleton phenotype was not reported	[260]	NA
FGF22	KO	Exon1 and part of Exon2	Germline	Viable	Normal skeletal patterns; decreased susceptibility to pharmacologically induced seizures	[212]	NA
	KO	Exons1-3	Germline	Viable	Normal skeletal patterns; decreased incidence of tumors by chemical induction	[261]	NA
FGF23	KO	Exon 1	Germline	Viable	Increased serum levels of phosphate, calcium and 1,25(OH) ₂ D, severe growth retardation with abnormal bone phenotype	[184]	NA
	KO-LacZ	Exons 1-3	Germline	Viable	Hyperphosphatemia and impaired skeletogenesis	[193]	NA
FGF23	KO-eGFP	Exon 1	Germline	Viable	Hyperphosphatemia and impaired skeletogenesis	[166]	NA
	OE	Transgene (CAG-hFGF23)	Various	Viable	Hypophosphatemia, low serum 1,25(OH) ₂ D level, and rachitic bone growth retardation	[179]	ADHR
FGF23	OE	Transgene (Col1a-hFGF23)	Osteoblastic lineage	Viable	Smaller body, decreased serum phosphate concentrations, low serum 1,25(OH) ₂ D level	[180]	ADHR
	OE	Transgene (Apoe3-hFGF23*R176Q)	Liver	Viable	Hypophosphatemia, low serum 1,25(OH) ₂ D level and rachitic bone	[181]	ADHR
FGF23	GOF (KI)	Knock in (R176Q-hFGF23)	Germline	Viable	Increased serum level of FGF23, hypophosphatemia and low serum 1,25(OH) ₂ vitamin D after receiving low-iron diets	[188,189]	ADHR

Abbreviations: ACH, achondroplasia; ADHR, autosomal dominant hypophosphatemic rickets; AS, Apert syndrome; BSS, Beare-Stevenson cutis gyrata syndrome; CATSHL, camptodactyly, tall stature and hearing loss; CKO, conditional knockout; CS, Crouzon syndrome; EKS, elbow knee synostosis; GOF, gain of function; KD, knockdown; KI, knock-in; KO, knockout; LMB, limb bud mesenchyme; LOF, loss of function; MS, Muenke syndrome; NA, not applicable; OE, overexpression; PS, Pfeiffer syndrome; SADDAN, severe achondroplasia with developmental delay and acanthosis nigricans; TD, thanatophoric dysplasia; XLH, X-linked hypophosphatemia.

give full attention of the exciting results from FGF23 studies, we encourage you to read the recently published review by Quarles and Bhattacharyya.^{31,204}

OTHER FGFS

In addition to the FGFS mentioned above, the roles of majority of these 22 FGFS are not defined in skeleton development and homeostasis. Researchers have generated knockout or CKO mouse models of these FGFS (Table 2). *Fgf3* knockout mice show a short, dorsally curled tail, caudal vertebrae and smaller body.²⁰⁵ Some mouse models show normal skeleton phenotypes, such as mice lacking FGF1, FGF5, FGF6, FGF7, FGF17 or FGF22.^{206–212} The skeleton phenotypes of knockout mice lacking FGF11–FGF16, or FGF20, are still not analyzed.^{213–219} The effect of these FGFS on bone development or homeostasis need be further studied.

CONCLUSIONS

Studies in human patients and mouse models with FGFS/FGFRs mutations have shown important roles of FGF signaling in skeletal development, genetic skeletal diseases and bone homeostasis. So, FGF/FGFR signalings will be attractive targets for treating bone related diseases. FGF/FGFR signals control the balance among skeletal cell growth, differentiation and apoptosis during development and adult homeostasis, as well as regulate systemic phosphate homeostasis. However, many unresolved issues still need to be explored.

Many studies have investigated the role of FGFRs in endochondral and intramembranous bone formation during development, but the effects of FGFRs on osteoclasts, especially on osteocytes, have not be clarified. Osteocytes are the most abundant and longest-living cells in the adult skeleton and have essential roles in bone homeostasis.^{220–221} Thus, uncovering the impact of FGFRs on osteocytes using osteocyte-specific Cre mice is critical.

Compensation, or crosstalk, may occur between different FGFRs during skeleton development. For example, conditional knock out of *Fgfr1* in mature osteoblasts leads to increased FGFR3 expression,¹⁵ whereas both cultured bone marrow stromal cells from *Fgfr3* null mice, or mice carrying gain-of-function mutation in FGFR3, have increased expression of FGFR1.^{83,85} Crossing between mouse strains harboring various FGFRs mutations is extremely important to elucidate the interactions between different FGFRs.

So far, only part of the 22 known FGF ligands have been shown to be essential for skeletal development, such as FGF8, FGF9 and FGF10. However, the mechanisms remain unclear because most of the knockout mice die before or after birth. Conditional deletion of these FGFS using bone cell-specific Cre mice is necessary to study their roles during

bone development. The function of other unexplored FGFS in skeletogenesis remains to be discovered. Furthermore, which FGFRs are the relatively specific receptors of these unexplored FGFS during bone development and metabolism are unknown. Crossing mouse strains harboring different FGFS mutations with FGFRs mutant mouse models is necessary to discover the interactions between FGFS and FGFRs in skeleton development and homeostasis.

Recently, studies have indicated that the bone is closely related with whole-organism physiology.²²² For example, bone can regulate energy metabolism, male reproduction and hematopoiesis.^{223–224} Some hormone secreted from other organs or tissues also have effect on bone, such as Leptin secreted by adipocyte.²²² In addition, systemic disease also influence skeleton such as CDK²²⁵ and inflammatory disease.^{226–227} The roles of FGF signaling in the effect of systemic diseases on bone or bone on whole-organism physiology remain unclear and need further exploration.

Conflict of Interest

The authors declare no conflict of interest.

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