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.

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. 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.

Various signaling molecules control the process of skeleton development, such as fibroblast growth factor (FGF), wingless-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 and induce the phosphorylation of tyrosine residues in the intracellular domain of FGFRs. The activated FGFRs recruit target proteins to its cytoplasmic tail and modifies these proteins by phosphorylation, 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 (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. At the epiphyseal growth plate, FGFR1 is expressed in perichondrium,
prehypertrophic and hypertrophic chondrocytes.\textsuperscript{9,11–12} FGFR1 is also expressed in osteoblasts and osteocytes (Table 1).\textsuperscript{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\textsuperscript{-/-}) embryos display severe growth retardation, and died prior to or during gastrulation because of intrinsic blocks in mesodermal differentiation.\textsuperscript{17–18} Deletion of the Ig domain IIIC of Fgfr1 (Fgfr1IIIC) leads to gastrulation defects resembling the Fgfr1\textsuperscript{-/-} alleles. However, mice with Fgfr1IIICb ablation are viable and fertile, suggesting that IIIC is the dominant isoform for the majority of FGFR1 functions in embryogenesis.\textsuperscript{19} Chimeras were generated by injecting Fgfr1\textsuperscript{-/-} 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.\textsuperscript{19–21}

Mice with targeted deletion of FGFR1 in all limb bud mesenchymal cells (via T (brachyury)-cre),\textsuperscript{22} or posterior limb bud mesenchyme (via Shh-cre)\textsuperscript{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.\textsuperscript{10} Li et al.\textsuperscript{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

![Signaling pathways activated by FGF/FGFR](image-url)
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<sup>Δfrs/Δfrs</sup>) 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

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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 |
|------------|----------|--------------------|-----------|--------------|---------------------|
| FGFR2      | Developing condensation | Perioskeletal cells, Osteoblasts in trabecular bone | Perichondrium, Chondrocytes | Mesenchymal cells in the suture | FGFR1, FGFR2, FGFR3c, FGFR4 |
| FGFR4      | Posterior AER at E10.5-11.0 | Cortical bone at embryonic stage | Perichondrium, Chondrocytes | Osteoblasts | FGFR2b, FGFR3c, FGFR4 |
| FGFR7      | Loose mesenchyme | Periosteum, Primary spongiosa | Perichondrium, Chondrocytes | Mesenchyme of suture in early craniofacial development stages | FGFR2c, FGFR3c, FGFR4 |
| FGFR8      | AER | Prehypertrophic and hypertrophic chondrocytes of growth plate, Perichondrium, Cartilage of the cranial base | Osteoblasts | FGFR2b, FGFR3c, FGFR4 |
| FGFR9      | AER, Developing condensation | Periosteum, Trabecular bone | Perichondrium, Chondrocytes | FGFR2b, FGFR3c |
| FGFR10     | Lateral plate mesoderm | Periosteum, Trabecular bone, Osteocytes | Chondrocytes | FGFR2b, FGFR3c |
| FGFR11     | Osteoblasts, Osteocytes in trabecular bone | Periosteum, Trabecular bone | Chondrocytes | FGFR2b, FGFR3c |
| FGFR12     | Center of the mesenchyme condensation | Periosteum, Trabecular bone | Chondrocytes | FGFR2b, FGFR3c |
| FGFR13     | Strictly in osteoblasts between the periosteal and endosteal layers | Periosteum, Trabecular bone | Chondrocytes | FGFR2b, FGFR3c |

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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 prechondrogenic 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 suture 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 suture39,49 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 FGFR2IIIc (Fgfr2c+/C342Y) (equivalent to mutation in human causes CS/PS), premature fusion of...
craniocutaneous features is accompanied by abnormal cranial sutures and increased proliferation of osteoprogenitor cells in the cranial sutures.\textsuperscript{53} Chen et al.\textsuperscript{51} also found decreased bone formation and premature closure of the coronal suture in \textit{Fgfr2}\textsuperscript{+/S250W} mice similar to phenotypes in human AS.\textsuperscript{51} However, increased apoptosis is responsible for premature fusion in \textit{Fgfr2}\textsuperscript{+/S250W} coronal suture.\textsuperscript{51} These results suggest that different activating mutations in FGFR2 result in craniosynostosis through distinct mechanisms.

\textit{Fgfr2illic}\textsuperscript{−/−} mice also show delayed differentiation and mineralization of the skull vault, and premature coronal suture due to decreased cell proliferation.\textsuperscript{54} The retarded ossification in \textit{Fgfr2illic}\textsuperscript{−/−} mice is correlated with the decreased osteoblast markers \textit{OP} and \textit{Cbfal}, which is emphasized by increased osteogenesis of Crouzon-like \textit{Fgfr2IIIc} ossification in \textit{C223}\textsuperscript{−/−} mice. This leads to premature fusion of cranial sutures.\textsuperscript{51} However, increased apoptosis is responsible for premature fusion in \textit{Fgfr2}\textsuperscript{+/S250W} coronal suture.\textsuperscript{51} These results suggest that different activating mutations in FGFR2 result in craniosynostosis through distinct mechanisms.

FGFR3

FGFR3 is first expressed in chondrocytes, differentiated initially from the core of the mesenchyme condensation.\textsuperscript{58} FGFR3 is expressed in reserve and proliferating chondrocytes as the epiphyseal growth plate is formed.\textsuperscript{12,58–59} Immunohistochemistry results have indicated that FGFR3 is also expressed in mature osteoblasts and in osteocytes.\textsuperscript{14} During calvarial bone development FGFR3 is expressed at low levels in sutural osteogenic fronts at the late stages (Table 1).\textsuperscript{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).\textsuperscript{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.\textsuperscript{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.\textsuperscript{64} Patients with SADDAN exhibit acanthosis nigricans and anomalies in the central nervous system, in addition to severe skeletal dysplasia.\textsuperscript{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.\textsuperscript{60,67–71} 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
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 promotes 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,1 FGFR4 is also highly expressed in rudimentary membranous bone and strictly localized in osteoblasts between the peristeal and endosteal layers (Table 1).92 Interestingly, Fgfr4-deficient mice are developmentally normal, but the Fgf3/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 chondrodysplasic phenotype of TgFGF2 mice.98 These results indicate the essential role of Stat1 in FGF-mediated regulation of epiphyseal growth plates. Fgf2-knockout (Fgf2<sup>−/−</sup>) mice have normal limbs. The normal skeleton in Fgf2<sup>−/−</sup> 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.\(^{75,101}\) 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.\(^{114,102}\) 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\(\alpha\) 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 (hmw, 21 and 22 kDa). FGF2lmw is secreted and activates FGFRs, whereas FGF2hmw remains intranuclear. Their roles in bone formation are largely unknown. Transgenic mice with targeted overexpression of FGF2lmw and FGF2hmw 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-FGF2hmw 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 FGF2hmw 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 Msx2-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 Msx2 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.\(^{120,127}\) 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.\textsuperscript{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).\textsuperscript{135} FGF9 is broadly expressed in different tissues including in AER, perichondrium/periosteum, chondrocytes of growth plate, as well as primary spongiosa.\textsuperscript{133–135}

Colvin \textit{et al.}\textsuperscript{136} generated \textit{Fgf9} knockout (\textit{Fgf9}\textsuperscript{−−/−}) mice and showed that deletion of \textit{Fgf9} alleles led to lethality at the neonatal stage mainly due to malformations of the lung, and causing male-to-female sex reversal.\textsuperscript{136–137} These results indicate that loss of \textit{Fgf9} function after mesenchymal condensation. The rhizomelia results from the loss of \textit{Fgf9} function after mesenchymal condensation. Similarly, transgenic mice, with overexpression of \textit{Fgf9} in chondrocytes (\textit{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.\textsuperscript{133} These seemingly inconsistent results between \textit{Fgf9} null and transgenic mice may result from distinct effect of \textit{Fgf9} on different stages of skeletogenesis.

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

\textit{Fgf9} is also expressed in the mesenchyme of suture in the early craniofacial development stages.\textsuperscript{115} By contrast to its promoting effects on osteogenesis in endochondral ossification, targeted overexpression of \textit{Fgf9} in cranial mesenchymal cells leads to a switch from intramembranous to endochondral ossification in mouse parietal bones, indicating that \textit{Fgf9} may regulate bone development by affecting the direction of mesenchyme differentiation.\textsuperscript{139}

Recently, missense mutations in \textit{Fgf9} have been identified to result in elbow-knee synostosis, premature fusion of cranial sutures in mice\textsuperscript{140} and multiple synostosis syndrome in humans.\textsuperscript{141} These data further suggest the important effect of \textit{Fgf9} on bone development.

However, the different impacts of \textit{Fgf9} on different stages of limb development and the direct effect of \textit{Fgf9} on adult bone homeostasis are still unclear. Targeted deletion of \textit{Fgf9} in different stages and cells using \textit{Fgf9} CKO mice\textsuperscript{142} 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,\textsuperscript{144–146} and the expression persists in the mesenchyme under AER after initial limb bud formation. FGF10 acts epistatically at the upstream of FGF8.\textsuperscript{145} Positive feedback exists between FGF8 and FGF10, which is essential for limb development.\textsuperscript{44} To define the role of FGF10, \textit{Fgf10} knockout (\textit{Fgf10}\textsuperscript{−−/−}) mouse strain was generated. These mice show complete absence of fore- and hindlimbs, and die after birth associated with complete absence of lungs.\textsuperscript{145–146} The limb bud formation in \textit{Fgf10}\textsuperscript{−−/−} embryos is initiated but outgrowth of the limb buds is impaired, while the clavicle formation is normal.\textsuperscript{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.\textsuperscript{11,147–148}

\textit{Fgf18} knockout (\textit{Fgf18}\textsuperscript{−−/−}) 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 \textit{Fgfr3}.\textsuperscript{147–148} Bone cultures of fetal mouse tibias treated with FGF18 show decreased bone length and hypertrophic differentiation of chondrocytes.\textsuperscript{87,149} These studies demonstrate the inhibitory effect of FGF18 in chondrogenesis. In contrast to the negative role of FGF18 in chondrogenesis found in \textit{Fgf18}\textsuperscript{−−/−} mice or FGF18-treated cultured bone, the proliferation and differentiation of primary chondrocytes and prechondrocytic ATDC5 cells are stimulated by FGF18 treatment in vitro.\textsuperscript{150} FGF18 also enhances BMP function and stimulates chondrogenesis in earlier stages of cartilage formation by suppressing noggin expression.\textsuperscript{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-1a-hydroxylase (1a-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 1a-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 Ftg23 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 Ftg23 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

| Parathyroid glands | Bone |
|-------------------|------|
| PTH               | FGF23 |
| FGF23             | PHEX |
| 1,25(OH)2D        | 1a-hydroxylase |
| NaPi-2a           | phosphate resorption |
| Renal tubules     | |

**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 1a-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 |
| FGFR1-deficient ES chimeras 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 |
| FGFR1-deficient ES chimeras 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 |
| FGFR1-deficient ES chimeras Exon 8 (IIb) | Germline | Viable | No obvious phenotype | [19] | NA |
| FGFR1-deficient ES chimeras Exon 9 (IIc) | Germline | Lethal | Gastrulation defects | [19] | NA |
| FGFR1-deficient ES chimeras 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 |
| CKO Exons 8-14 Shh-cre (posterior LBM cells) | Die at birth | Normal limb bud size, missing digit 3 | [10,229] | NA |
| CKO Exons 8-14 Ap2-Cre (progress zone of the mouse limb at E10.5) | Die at birth | Abnormal development of the anterior digits | [24] | NA |
| CKO Exons 8-14 Hoxb6-Cre (lateral plate mesoderm of E8.5) | Die at birth | Severe abnormalities in autopod formation in hindlimbs | [24] | NA |
| CKO Exons 8-15 Col2a1-cre (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 |
| CKO Exons 8-15 Col1-cre (differentiated osteoblasts) | Viable | Increased bone mass, accelerated osteoblast differentiation and mineralization, impaired osteoclast activity | [15,230] | NA |
| CKO Exons 8-15 LysM-cre | Viable | Increased bone mass, impaired osteoclast formation and activity | [29] | NA |
| CKO Exons 8-15 OC-cre | Viable | Increased bone mass | Su et al. unpublished data | NA |
| DN Transgene (Tyrp1-FGFR1*IIc) | Retinal pigment epithelium | Die at E11.5 | Developmental delay, mesodermal migration and patterning defects, craniorachischis and posterior truncations | [25] | 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) |
| 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-LacZ | Exons 7–9 (Entire Ig III) | Germline | Die at E10–11 | Failure of limb bud initiation and placenta formation                                      | [44,45] | NA                          |
|       | KO    | Exon 8 (IIIb) (A translational stop codon inserted into exon 9) | Germline | Die at birth  | Impaired limb outgrowth, severe dysgenesis of multiple organs                            | [233]   | NA                          |
|       | KO    | Exon 9 (Iic) (Resulting in a GOF mutation associated with exon switching within the Fgfr2 gene) | 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 (IIIb) | CMV-Cre (germline) | Die at birth | Defects of limb outgrowth and branching morphogenesis                                      | [234]   | NA                          |
|       | CKO   | Exon 9 (Iic) (Resulting in a GOF mutation associated with exon switching within the Fgfr2 gene) | ZP3-Cre (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 | Dermo1-Cre (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-Fgfr2) | EIIa-Cre (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 basiscranium, 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 (Iic) (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 (Iic) 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                         |
| 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               |
| FGFR3  | KO     | From Ig-like domain II to the transmembrane domain | Germline | Viable   | Bone overgrowth, defective bone mineralization and osteopenia, early arthritids, 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                            |
| OKO    | Exons 9–10 | EIIa-Cre | Viable | Increased length of long bone and decreased bone mineral density           | [239] | NA                            |
| GOF (K1) | Exon 7 (P244R) | Germline | Viable | Abnormal craniofacial morphology                                           | [240] | MS (P250R)                    |
| GOF (K1) | Exon 9 (Y367C) | Germline | Viable | Skeletal dysplasia more severe than ACH                                    | [241] | TD I (Y373C)                  |
|        |        | (die at 6–8 weeks after birth) |         |          |                                                                           |          |                               |
| GOF (K1) | Exon 10 (S365C) | Germline | Viable | Skeletal dysplasia more severe than ACH                                    | [72] | TD I                          |
| GOF (K1) | Exon 10 (G369C) | Germline | Viable | Macroprephal and shortened limbs due to retarded endochondral bone growth and premature closure of cranial base synchondroses | [70] | ACH (G375R)                   |
| GOF (K1) | 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 (K1) | K644E cDNA knock-in | Germline | Viable | Retardation of bone growth, macrocephaly and shortening of the long bones resembling ACH patients | [73] | ACH                           |
| GOF (K1) | Exon 15 (K644E) | Germline | Neonatal lethality | Die within few hours after birth, skeletal dysplasia more severe than ACH | [71] | TD II (K650E)                 |
| GOF (K1) | 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                            |
| FGFR3/ FGFR4 | Double KO | Exon 8 (G385R) | Germline | Viable | Skeleton phenotype not reported                                             | [244] | NA                            |
| FGFR4  | KO     | Exon1    | Germline          | Viable   | Neonatal growth retardation, lung abnormalities                           | [93] | NA                            |

Continued
| Gene Model | Gene | Exon | Cre line (tissue) | Survival | Phenotype | Reference | Related human skeleton disease |
|------------|------|------|-------------------|----------|-----------|-----------|-------------------------------|
| KO         | FGF2 | Exon1 (all three isoforms) | Germline | Viable | Impaired cerebral cortex development, blood pressure regulation | [245] | NA |
| KO         | FGF2 | Exon1 (all three isoforms) | Germline | Viable | Decreased bone mass, decreased vascular smooth muscle contractility, low blood pressure and thrombocytosis | [95,100] | NA |
| KO         | FGF2 | Exon1 (All three isoforms) | Germline | Viable | Delayed wound healing and neuronal defects and impaired development of the cerebral cortex | [99] | NA |
| KO         | FGF2 | 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         | FGF2 | 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 |
| KO         | FGF2 | Heterozygous (Fgf2+/−) | Exon1 | Viable | Decreased bone mass and bone formation | [104] | NA |
| OE         | FGF2 | 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         | FGF2 | 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         | FGF2 | Transgene (3.6 kb Col1a-HMW FGF2-IRES-GFPsaph) | Immature and mature osteoblast lineage | Viable | Dwarfism, decreased BMD, osteomalacia, increased FGF23 level and hypophosphatemia | [109,110] | similar to XLH |
| KO         | FGF3 | Exon1b (leaky expression of the mutant Fgf3) | Germline | Die in the early postnatal period | A short, dorsally curled tail and caudal vertebrae, smaller body, Inner ear defects | [205] | NA |
| KO         | FGF3 | Exons1b-3 | E11.5-Cre (germline) | Viable | Shortened, thickened and curved tail, normal inner ears | [248,249] | NA |
| KO         | FGF3 | Exon 2 | CMV–cre (germline) | Viable | Short, curly tails, abnormal olf morphologies | [250] | NA |
| KO         | FGF3 | Exon 2 | Not used for skeleton research | Viable | Not used for skeleton research | [251] | NA |
| OE         | FGF3/4 | 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 |
| KO         | FGF4 | Exon 1 | Disrupted prior to limb bud initiation | Died at E5.0 | Severely impaired proliferation of the inner cell mass | [113] | NA |
| KO         | FGF4 | Exons 1–3 | Rarb/Cre (developing forelimb region) | Viable | Normal forelimbs and hindlimbs | [111,126] | NA |
| KO         | FGF5 | Exons 2–3 | MSX2-cre | Viable | Normal forelimbs and hindlimbs | [112] | NA |
| KO         | FGF6 | Exon 1 | Germine | Viable | Abnormally long hair, impaired skeletal muscle | [207] | NA |
| KO         | FGF7 | Exon 1 | Germine | Viable | No abnormal phenotype of skeleton detected | [208] | NA |
| KO         | FGF7 | Exon 1 | Germine | Viable | No abnormal phenotype of skeleton detected | [209] | NA |
| Gene Model | Exon | Cre line (tissue) | Survival | Phenotype | Reference | Related human skeleton disease |
|------------|------|------------------|----------|-----------|-----------|--------------------------------|
| FGF8 KO    | Exons 2–3 + neo | Germine | Lethal | Early embryonic lethality before limb development | [123,124] | NA |
| FGF8 KO    | Exons 2–3 | α-actin-cre (early embryo) | Lethal | Early embryonic lethality | [123,124] | NA |
|            | Exons 2–3 | MSX2-cre (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 |
|            | Exons 2–3 | Rarb-Cre (developing forelimb region) | Not mentioned | Severe forelimb deformity including absence of radius and first digit | [125,126] | NA |
| FGF9 KO    | Exon 1 | Germine | Die at birth | Lung hypoplasia, male-to-female sex reversal, inner ear morphogenesis defect, slightly smaller body, short proximal skeletal | [135–137,253] | NA |
| FGF9 Heterozygous (Fgf9+/−) | Exon 1 | Germine | Viable | Reduced bone regeneration, impaired neovascularization and decreased cell proliferation | [254] | NA |
|            | Exon 1 | Nestin Cre (germline) | Die at birth | Lung hypoplasia, skeleton phenotype not mentioned | [142] | NA |
|            | Exon encoding the ATG translational start site | Germline | Perinatal lethality | Complete absence of both fore- and hindlimbs, pulmonary branching morphogenesis was completely disrupted | [145] | NA |
| FGF10 KO   | Exon 1 | Germline | Die after birth | Complete truncation of the fore- and hindlimbs, normal clavicles, lung defect | [146] | NA |
| FGF11 KO   | Exon 2 | Germline | Viable | Skeleton phenotype was not analyzed | [251] | NA |
| FGF12 KO   | Exon 2 | Germline | Viable | Skeleton phenotype was not analyzed | [213] | NA |
| FGF13 KO   | Exons 2–3 | Germline | Viable | Skeleton phenotype was not analyzed | [214] | NA |
| FGF14 KO-LacZ | Exon 2 | Germline | Viable | Skeleton phenotype was not analyzed, developed ataxia and a paroxysmal hyperkinetic movement disorder; reduced responses to dopamine agonists | [215] | NA |
| FGF15 KO   | Exon 3 | Germline | Die at E13.5–P21 | Skeleton phenotype was not analyzed, enhanced bile acid synthesis and contracted gallbladder | [216] | NA |
| FGF16 KO-LacZ | Exons 2–3 | Germline | Viable | No bone phenotype was analyzed, decreased proliferation of embryonic cardiomyocytes | [217] | NA |
| FGF17 KO   | Exons 1a–1b | Germline | Viable | Normal skeletal patterns, abnormal cerebellar development and social behaviors | [210,211] | NA |
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, increased 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                             |
|        |        |               |                   |                        |                                                                           |                    |                                |
| Heterozygous | | Exon 3 | Germline          | Viable               | Reduced bone regeneration                                                  | [258]              | NA                             |
| KO     | Exon 1 | Germline      | Viable            |                        |                                                                           | [259]              | NA                             |
| FGF20  | KO-LacZ| Exons 2, part of exon 1 and exon 3 | Germline          | Viable               | No bone phenotype was analyzed, deafness                                  | [218]              | NA                             |
|        | KO     | Exons 1–3     | Germline          | Viable               | Greater body and tibia growth after food restriction                       | [157,161]          | NA                             |
| KO (LacZ) | Exons 1–3 | Mox-cre (Germline) | Viable               |                        | Skeleton phenotype was not reported                                          | [156]              | NA                             |
| CKO    | Exons 1–3 | Meox-cre (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                             |
|        | Transgene | Apoe-hFGF21 | Liver             | Viable               | Skeleton phenotype was not reported                                         | [260]              | NA                             |
| FGF22  | KO     | Exon 1 and part of Exon 2 | Germline          | Viable               | Normal skeletal patterns; decreased susceptibility to pharmacologically induced seizures | [212]              | NA                             |
| KO     | Exons 1–3 | 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)2D, severe growth retardation with abnormal bone phenotype | [184]              | NA                             |
| KO-Lacz | Exons 1–3 | Germline      | Viable            |                        | Hyperphosphatemia and impaired skeletogenesis                              | [193]              | NA                             |
| KO-eGFP | Exon 1 | Germline      | Viable            |                        | Hyperphosphatemia and impaired skeletogenesis                              | [166]              | NA                             |
| OE Transgene | CAG-hFGF23 | Various | Viable            |                        | Hyperphosphatemia, low serum 1,25(OH)2D level, and rachitic bone, growth retardation | [179]              | ADHR                           |
| OE Transgene | Apoe3-hFGF23 | Osteoblastic lineage | Viable            |                        | Smaller body, decreased serum phosphate concentrations, low serum 1,25(OH)2D level | [180]              | ADHR                           |
| GOF (KI) | Knock in (R176Q-hFGF23) | Germline | Viable |                        | Increased serum level of FGF23, hypophosphataemia 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, camptotactyly, tall stature and hearing loss; CKO, conditional knock out; 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.21,204

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.213–217 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 FGFR1.33,85 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 FGFRs 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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Bone Research (2014) 14003

N Su et al

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