BMP signalling in skeletal development, disease and repair

Valerie S. Salazar, Laura W. Gamer and Vicki Rosen

Abstract | Since the identification in 1988 of bone morphogenetic protein 2 (BMP2) as a potent inducer of bone and cartilage formation, BMP superfamily signalling has become one of the most heavily investigated topics in vertebrate skeletal biology. Whereas a large part of this research has focused on the roles of BMP2, BMP4 and BMP7 in the formation and repair of endochondral bone, a large number of BMP superfamily molecules have now been implicated in almost all aspects of bone, cartilage and joint biology. As modulating BMP signalling is currently a major therapeutic target, our rapidly expanding knowledge of how BMP superfamily signalling affects most tissue types of the skeletal system creates enormous potential to translate basic research findings into successful clinical therapies that improve bone mass or quality, ameliorate diseases of skeletal overgrowth, and repair damage to bone and joints. This Review examines the genetic evidence implicating BMP superfamily signalling in vertebrate bone and joint development, discusses a selection of human skeletal disorders associated with altered BMP signalling and summarizes the status of modulating the BMP pathway as a therapeutic target for skeletal trauma and disease.

The human skeleton includes over 200 bones and 340 joints, as well as an intricate network of tendons, ligaments and cartilage. During development and postnatal life, bone and joint health is profoundly affected by genetics and environmental factors such as nutrition and exercise. Unsurprisingly, the skeletal system is a major site of human disease. As the name implies, bone morphogenetic proteins (BMPs) were originally discovered by their ability to induce new bone formation[1–4]; accordingly, recombinant human BMPs have been exploited as osteoinductive agents to repair bone defects in clinical settings[5]. However, our current understanding of BMP superfamily molecules further establishes these signals as mediators of normal skeletogenesis as well as the underlying aetiology of several debilitating skeletal pathologies including fibrodysplasia ossificans progressiva (FOP)[6], Marfan syndrome[7], Loeys–Dietz syndrome[8] and osteoarthritis[9,10]. In this Review, we describe BMP superfamily signalling in the context of skeletal development and joint morphogenesis, with the premise that the pathway is poised as a promising therapeutic target for treating skeletal trauma and diseases beyond bone repair. We open with a historical account of how BMPs were discovered, present a phylogenetic analysis of key molecules in the BMP signalling pathway and summarize fundamental BMP family signalling mechanisms in vertebrates. We then discuss developmental skeletogenesis, focusing on the genetic evidence from mice and humans supporting a decisive role for the BMP pathway in skeletal development and disease and conclude by summarizing nodes of the pathway that are currently or potentially accessible as therapeutic targets for clinical medicine.

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Historical perspective

Marshall Urist practiced orthopaedic surgery and conducted scientific research at the University of California, Los Angeles Medical School, USA, for nearly half of the twentieth century. At the time of his practice, the therapeutic potential of applying shavings from healthy bone to heal major bone defects had long been recognized in orthopaedic settings[11], although the mechanism for repair was unknown. In the 1960s, Urist identified an interfibrillar protein complex[1] in demineralized rabbit bone able to induce calcified cartilage from minced muscles *in vitro*[2] and bone formation at nonskeletal sites in rats[3]. Urist named this factor bone morphogenetic protein. Although initially ignored, Urist's work was eventually reproduced and published by Nobel laureate Charles Huggins[12], sparking intense efforts to identify and purify bone morphogenetic protein. The challenging purification of BMPs from bone matrix took many years and, in the end, researchers were unable to purify a homogeneous BMP[13,14]. Human BMPs were finally cloned in 1988, and it was then realized that the BMP activity Urist first identified consisted of multiple individual

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Key points

- Phylogenetic analysis indicates that the bone morphogenetic protein (BMP) pathway is ancient and highly conserved across the animal kingdom
- Gene duplication and divergence has created a diverse matrix of BMP ligand–receptor pairs that achieve sophisticated control of signalling through variable activity profiles and functional redundancy
- Members of the BMP superfamily affect almost all aspects of bone, cartilage and joint biology
- Altered BMP signalling is a major underlying cause of human skeletal disorders
- Modulation of BMP signalling is emerging as a promising therapeutic strategy for improving bone mass and bone quality, ameliorating diseases of skeletal overgrowth and repairing damage to bones and joints

related gene products[4]. Since that time, recombinant human BMP2 and BMP7 have been used in orthopaedic applications, where enhancing bone repair by activating BMP signalling has become standard practice in treating non-union fractures, spinal surgeries and oral maxillofacial procedures[5,15].

Signalling mechanisms of the BMP pathway

Essential components

The BMP pathway is at least 1.2–1.4 billion years old, emerging in the evolutionary record with multi-cellular animals[16]. Consistent with the role of transmitting information between cells, BMP signalling coordinates many developmental processes including body axis determination[17], germ layer specification, tissue morphogenesis and cell-fate specification. Phylogenetic analysis reveals that protein sequences for ligands, receptors and SMADs of the BMP pathway are highly conserved across distant species in the animal kingdom such as mice, flies and worms[18]. Full-length protein sequences of human and fly orthologues also exhibit considerable similarity[19,20] (FIG. 1), and this evolutionary conservation is particularly striking in the amino acid sequence of active mature signalling proteins produced after post-translational processing of prepeptide and propeptide domains[21,22]. In fact, striking examples of cross-species activity have been documented in which fly orthologues of BMP2 and/or BMP4 and BMP7 (Dpp and Gbb, respectively) can successfully induce endochondral bone formation when implanted in mammals[23].

At the most empirical level, BMP signalling relies on a source of secreted ligands and a target cell expressing type I and type II BMP receptors. Ligand-binding events activate a complex array of downstream intracellular mediators including, most notably, the canonical SMAD pathway[21,24]. Although weak transcription factors on their own, SMADs are potent regulators of gene expression via their ability to recruit chromatin-remodelling machinery and tissue-specific transcription factors to the genomic landscape[25–28]. Despite the seemingly simple nature of this signal transduction cascade, >30 secreted ligands, seven type I receptors, five type II receptors and eight SMADs have been identified in humans. Gene expression programs initiated by BMP superfamily signals are therefore highly diverse and tailored by factors such as ligand identity and concentration, the type I and type II receptor profile on the target cell, the repertoire of tissue-specific transcription factors that define which SMAD-dependent gene targets are regulated[27] and the status of the epigenetic landscape[26]. The number of genes regulated by any single BMP superfamily ligand can therefore be either very low or very high, permitting the system to accommodate distinct transcriptional requirements of both quiescent stem cells and differentiated cells with complex physiological activity.

Ligands. This extensive ligand family includes BMPs, growth/differentiation factors (GDFs), transforming growth factors (TGFs), activins, Nodal, and anti-Müllerian hormone (AMH). Collectively, these molecules are typically referred to as the TGF-β superfamily, although this terminology is based on the order of their discovery as opposed to phylogenetic analysis, which identifies BMP2 as the founding family member[22]. Whereas BMPs were discovered as a result of their osteoinductive qualities, activins and inhibins were originally discovered by their opposing control of follicle-stimulating hormone production[29], and TGF-βs were first reported as secreted factors that conferred malignancy on cells via autocrine induction[30]. Aside from sequence similarity, these ligands can be further organized into three groups on the basis of preferred receptor usage and SMAD1/5/8 versus SMAD2/3 signalling activity (FIG. 2). In general, ligands are initially translated as preproproteins, which facilitates targeting to the secretory pathway for proteolytic cleavage and enables noncovalent assembly into fully active dimers upon secretion via conserved cystine knot motifs[31,32]. Except for Nodal, proteolytic activation and dimerization is essential for signalling[33]. Both homodimers and heterodimers exhibit biological activity[34] that is well typified by activins, which can form active homodimers or heterodimers of activin βA, activin βB, activin βC or activin βE subunits. Activins can alternatively dimerize with inhibin α, and although this dimer retains receptor-binding activity, it constitutes a non-signal-generating ligand. Most ligands exhibit local paracrine activity, although some BMPs, activins, TGF-βs and GDFs are thought to circulate and exert systemic effects[35–39].

Receptors. Type I and type II BMP receptors are the only known class of transmembrane cell surface receptors in humans with serine/threonine kinase activity. A mature receptor signalling complex requires one ligand dimer, two type I receptors and two type II receptors (FIG. 2). Several mechanisms are utilized to form activated ligand:receptor complexes, which affect the specificity of ligand-receptor pairing[40,41] and competition by distinct ligands for shared receptors[42]. Whereas type II receptors are constitutively active, type I receptors encode a Gly/Ser-rich domain that must be phosphorylated by a type II receptor to activate intrinsic kinase activity (FIG. 2) and subsequently stimulate the recruitment and phosphorylation of the essential downstream pathway mediators known as receptor-activated SMADs (R-SMADs)[43] (FIG. 2).

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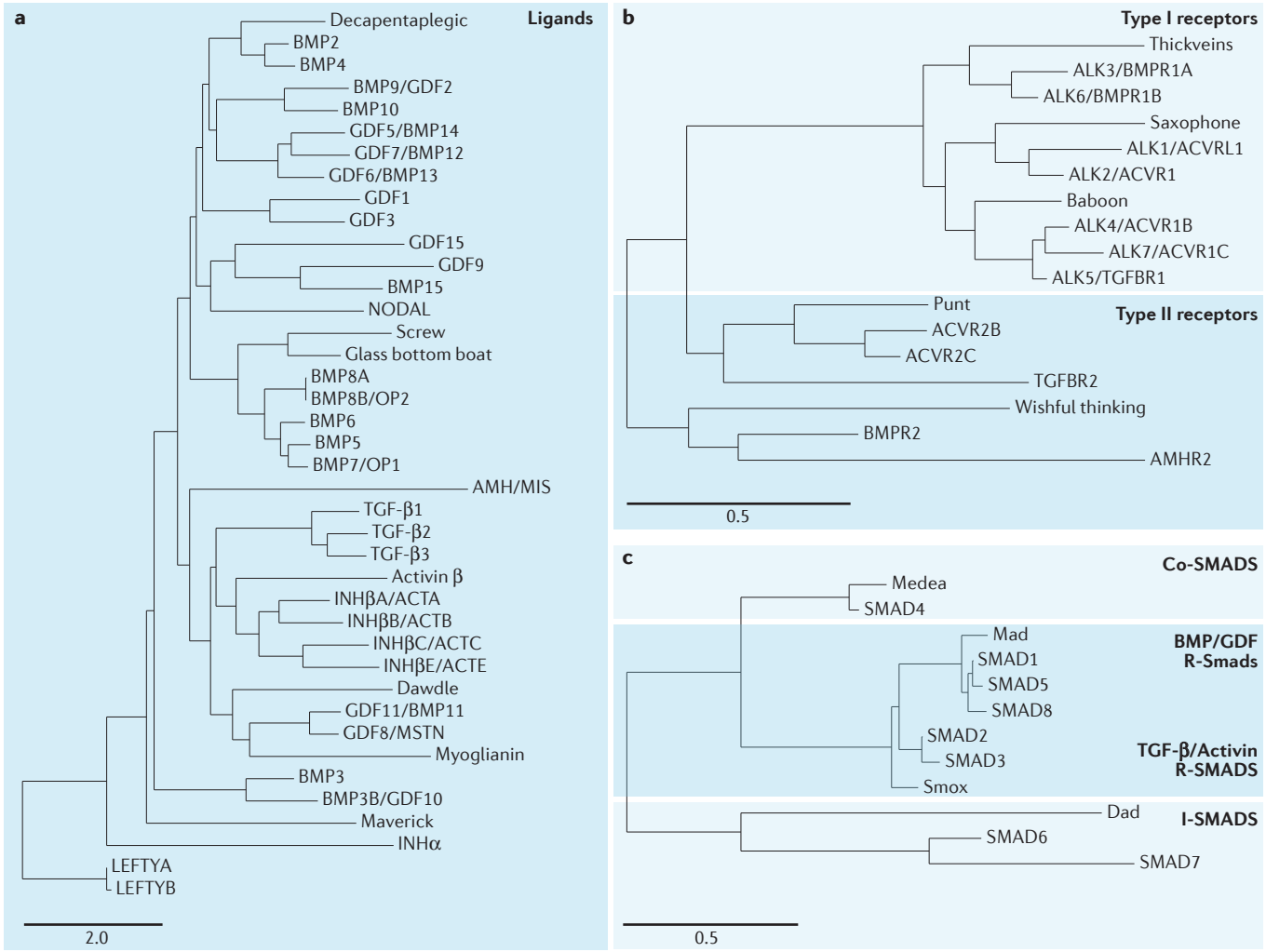

Figure 1 | **Phylogenetic analysis of BMP superfamily molecules.** Protein sequences from flies and humans were aligned to assess evolutionary relationships between bone morphogenetic protein (BMP) superfamily molecules. Human proteins are designated in all capital letters; only the first letter of fly proteins is capitalized. For ligands, preproprotein sequences were used for alignments. The longest known isoform of each molecule was used when applicable. Molecules are grouped into **a** | ligands, **b** | type I and type II receptors and **c** | SMADs. Branch lengths are drawn to scale; the scale bar indicates to the number of amino acid substitutions per site between two compared sequences. ACV, activin; ACVR, activin receptor; ALK, activin receptor-like kinase; AMH, anti-Müllerian hormone; AMHR2, AMH receptor-2; BMPR, BMP receptor; GDF, growth/differentiation factor; INHβ, inhibin β; co-SMAD, common SMAD; I-SMAD, inhibitory SMAD; R-SMAD, receptor-activated SMAD; TGF-β, transforming growth factor β; TGFBR, TGF-β receptor.

SMADs. SMADs are homologues of *Drosophila melanogaster* Mad proteins (mothers against decapentaplegic) and *Caenorhabditis elegans* SMA proteins (small body size), and encode cytoplasmic proteins required for responsiveness to BMP superfamily ligands[44]. SMADs are modular in structure, with many highly conserved motifs. Among these, the *N*-terminal MH1 domain contains a sequence-selective[45] DNA-binding motif[46] and nuclear localization signal[47] essential for SMAD-dependent effects on gene expression in response to ligand-binding events[48]. A conserved L3 loop motif mediates direct binding between R-SMADs and activated receptors and determines SMAD1/5 versus SMAD2/3 pairing specificity[49]. A series of serine/threonine residues in the linker domain enables SMADs to receive regulatory inputs from a variety of intracellular kinase cascades including inhibitory regulation by mitogen-activated protein kinase (MAPK)[50] and glycogen synthase kinase 3β (GSK3β)[51,52], facilitating integration of BMP signals with other pathways including fibroblast growth factor (FGF) and WNT. The C-terminus of SMADs contains serine/threonine (Ser/Thr) residues directly phosphorylated by type I receptors, as well as protein–protein interaction domains that mediate R-SMAD/SMAD4 trimerization[53] (FIG. 2). Activated SMAD complexes translocate to the nucleus where they target the genome via consensus SMAD-binding motifs, integrate with tissue-specific transcription factors and recruit chromatin remodelling machinery[25–28] (FIG. 2).

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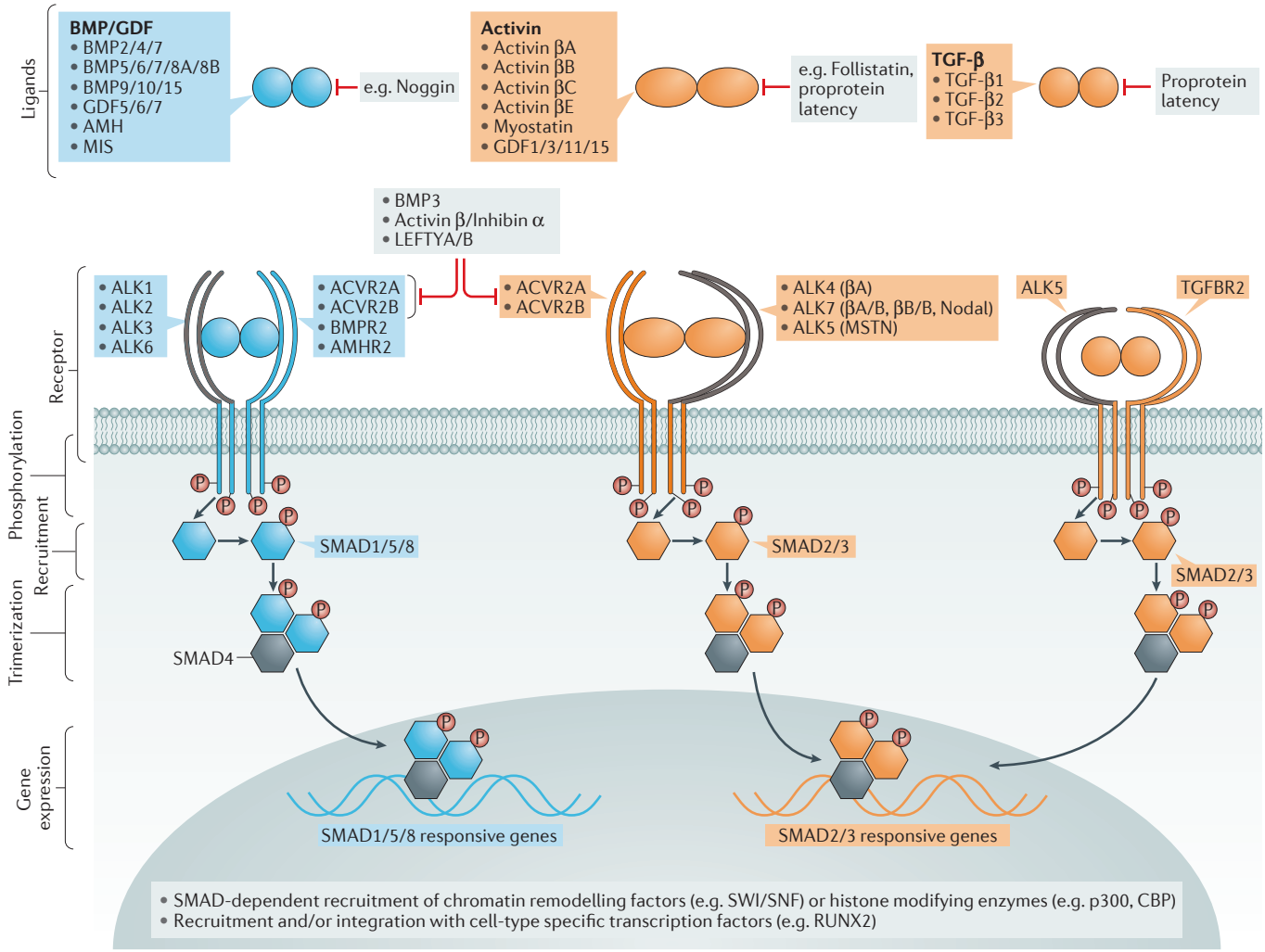

Figure 2 | **Fundamental mechanisms of canonical BMP superfamily signalling.** Over 30 bone morphogenetic protein (BMP) superfamily ligands have been discovered in humans. Most are secreted as mature disulfide-linked dimers, with the exception of TGF-β1, TGF-β2 and TGF-β3, which can be secreted in a latent form and require proteolytic activation. BMPs signal through a multimeric cell surface complex consisting of two type I receptors and two type II receptors. Type I and type II BMP receptors are single pass transmembrane proteins with an intracellular serine/threonine kinase domain. After ligand binding, type II receptors phosphorylate (P) the type I receptors. Activated type I receptors recruit and phosphorylate pathway-specific R-SMADs (SMAD1, SMAD5 and SMAD8 (blue pathway), and SMAD2 and SMAD3 (orange pathway)), which can form trimers with SMAD4 and translocate to the nucleus. SMADs have intrinsic DNA-binding activity and are able to regulate gene expression by recruitment of chromatin-remodelling machinery and integration with tissue-specific transcription factors. SMAD8 is also known as SMAD9. The pathway can be antagonized by many mechanisms including neutralization of ligands by secreted traps such as noggin or follistatin, secretion of latent ligands bound to their propeptides, or via titration of receptors by nonsignalling ligands such as BMP3, activin β/inhibin α dimers or LEFTY monomers. ACVR, activin receptor; ALK, activin receptor-like kinase; AMH, anti-Müllerian hormone; AMHR2, AMH receptor 2; BMPR, BMP receptor; GDF, growth/differentiation factor; TGF, transforming growth factor; TGFBR, TGF-β receptor.

Receptor/SMAD usage profiles

Ligand-receptor pairing specificity (reviewed elsewhere[54]) is summarized in FIG. 2. TGF-βs use the type I (ALK5) and type II (TGFBR2) TGF-β receptors to activate the SMAD2/3 pathway (FIG. 2, orange pathway). By contrast, BMPs and GDFs exhibit broad receptor usage patterns to activate the SMAD1/5/8 pathway (FIG. 2, blue pathway). Ser/Thr-protein kinase receptor R3 (ALK1), activin receptor type-1 (ALK2), BMP receptor type-1A (ALK3) and BMP receptor type-1B (ALK6) can all function as type I BMP and GDF receptors; BMP receptor type-2 (BMPR2), activin receptor type-2A (ACVR2A) and ACVR2B serve as type II receptors. Nodal, GDF8 and GDF11 activate SMAD2/3 via ALK4, ALK5, or ALK7 type I receptors and the ACVR2A and ACVR2B type II receptors. Activins utilize ALK4 (βA/βA) and ALK7 (βA/βB and βB/βB) for type I receptors, and ACVR2A and ACVR2B for type II receptors (FIG. 2). Importantly, activins can also bind to ALK2, but these complexes do not normally signal[55].

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Pathway antagonism

The BMP pathway is subject to many levels of regulatory activity, including propeptide latency, antagonism by secreted receptors and ligands, receptor trafficking and negative intracellular feedback by SMAD6/7 (REFS 54,56,57). As examples, noggin[58], gremlin[59] and follistatin[60] are secreted antagonists that are expressed in skeletal tissues and bind to distinct subsets of BMPs, GDFs and/or activins to titrate active ligands out of the extracellular environment[61,62] (FIG. 2). GDF8, GDF11 and TGF-βs can be secreted noncovalently attached to their prodomain, requiring additional processing to be activated from latency[63] (FIG. 2). Receptor availability can be regulated by BMP3 (REF. 64), LEFTYA/B monomers[65] and activin β/inhibin α heterodimers, which occupy but do not activate ACVR2A and/or ACVR2B (FIG. 2). This regulation dampens activin as well as BMP signalling, as ACVR2A and ACVR2B are shared receptors for these two ligand subtypes. Inside the cell, BMP and TGF-β signalling initiate negative feedback by transcriptional upregulation of SMAD6 and SMAD7, which are also known as the inhibitory SMADs (I-SMADs). By interacting with cytoplasmic domains of cell surface receptors, SMAD6 can sterically interfere with R-SMAD phosphorylation and recruit E3 ubiquitin ligases to mark signalling machinery for degradation[66–70]. Although long considered an intracellular signalling mediator of the canonical BMP pathway, new evidence suggests that SMAD8 (also known as SMAD9) is hypermorphic relative to SMAD1 and SMAD5, and so attenuates canonical BMP signalling[71]. Additional details on signalling and regulatory mechanisms can be found elsewhere[21,24,56,72].

Genetics of the BMP pathway

Developmental skeletogenesis

A skeleton with articulated joints appeared >400 million years ago in Cambrian bony fishes. In modern day mammals, the axial skeleton includes the skull, ossicles of the middle ear, hyoid bone, ribs, sternum and vertebrae. The appendicular skeleton comprises the pelvic and pectoral girdles and bones in the limbs. All bones are formed during development from three embryonic lineages: neural crest, paraxial mesoderm and lateral plate mesoderm. Some bones, such as those found in the skull, form by intramembranous ossification, in which migratory cells from the neural crest and paraxial mesoderm condense into sheet-like structures, differentiate into bone-forming cells called osteoblasts and produce mineralized tissue. Most bones, however, form by endochondral ossification, where a cartilage template produced by chondrocytes is segmented by joints, populated by haematopoietic progenitors during a primary wave of vascularization, remodelled by monocyte-derived resorbing cells called osteoclasts, and finally converted into bone by osteoblasts. The development of endochondral bones, therefore, requires the coordination of signals from several distinct cell types developing within the cartilage rudiment[73] (FIG. 3).

Before bone and joint formation, the mesenchymal progenitor pool in the emerging limb bud must first undergo considerable expansion and patterning[74]. Lineage tracing analysis reveals that most, if not all, connective tissue cell types in the limb skeleton and some structures in the cranial vault arise from *Prx1*$^+$ progenitors[75] (Prx1 is also known as Prrx1; FIG. 3a). Accordingly, *Prx1–Cre*[75] has become a useful tool for conditionally ablating genes selectively in the limb bud mesenchyme (FIG. 4a), without the embryonic lethality resulting from global-deficiency, such as is the case with *Bmp2* (REF. 76). *Prx1*$^+$ progenitors are highly responsive to BMP signalling as limb bud outgrowth and patterning are disrupted in mice lacking *Alk3* (REF. 77), and severely impaired in mice with *Prx1–Cre*-mediated single deletion of *Smad4* or compound deletion of *Alk2, Alk3* and *Alk6* (REFS 78,79). However, limb bud outgrowth ensues normally in mice with single or compound deletions of *Bmp2, Bmp4* and *Bmp7* (REFS 80–83), and is only modestly impaired by global compound deletions of *Gdf5* and *Gdf6* or *Gdf5* and *Bmp5* (REFS 84,85), which suggests that BMP signals essential for limb bud outgrowth are normally provided by multiple BMP-like ligands. Both genetic methods as well as classic 'cut and paste' experiments have further demonstrated that tissue nonautonomous BMP signals essential for limb bud patterning and digit specification emerge from ectodermal cells in the limb bud organizing centre known as the apical ectodermal ridge (AER)[86]. Expression of *Msx2* is highly enriched in the AER[87] and *Msx2–Cre* has been used to make selective compound deletion of *Bmp2*, *Bmp4* and *Bmp7* (REF. 88). Consistent with a cell autonomous role for BMP signalling in the mesenchyme, loss of *Bmp2*, *Bmp4* and *Bmp7* in the AER (*Bmp2; Bmp4; Bmp7; Msx1–Cre*) has no effect on limb bud outgrowth, but instead leads to loss of the AER and striking defects in digit patterning[88]. Digit patterning is also affected by mesoderm-derived BMP signalling as overexpression of gremlin in the limb bud mesenchyme mediates specification of too few versus too many digits, depending on the timing of induction[89].

Although the confluence-sensing mechanism remains unclear, the expanding progenitor pool eventually reaches a critical mass and triggers condensation, which is required for entry of progenitors into endochondral differentiation programs and imparts shape on presumptive skeletal elements. As these cells become specified to the chondrogenic lineage, they upregulate *Col2a1* and *Agc1* (FIG. 3b), and begin depositing a cartilage matrix. Cells at the innermost regions of the condensation upregulate *Col10a1* as they differentiate into hypertrophic chondrocytes (FIG. 3c).

Chondrocyte hypertrophy at the centre of the mesenchymal condensation is coupled to vascular invasion (FIG. 3c), which delivers an influx of haematopoietic cells that give rise to osteoclasts that excavate the cartilage template, and other constituent cells that populate the newly formed marrow space (FIG. 3d). Importantly, vascular invasion further acts to bisect the endochondral structure, creating two inversely stratified and distally opposed growth plates that establish a longitudinal axis of growth[90,91]. Longitudinal growth is enforced by molecular crosstalk between stratified layers of immature and hypertrophic chondrocytes within each growth plate[92–94] (FIGS 3d,3e).

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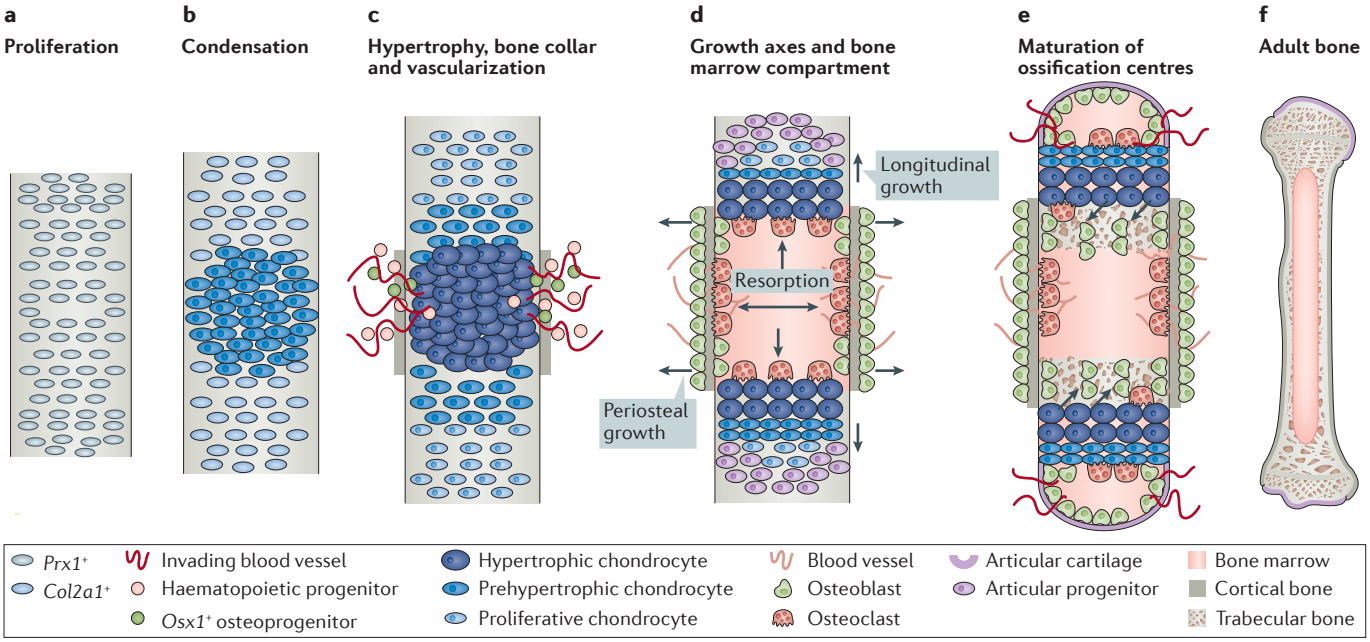

Figure 3 | **Developmental skeletogenesis.** Longitudinal views depicting key steps of endochondral bone formation in mouse limbs. **a** | *Prx1*$^+$ progenitors from lateral plate mesoderm proliferate to populate the emerging limb bud. **b** | Cells nearest the centre undergo mesenchymal condensation, express *Col2a1* as they enter a chondrogenic differentiation program, and deposit a cartilage template. **c** | to **d** | Differentiating cells upregulate *Col10a1* as they become hypertrophic, which triggers local formation of a bone collar and vascularization of the cartilage template. Invading blood vessels deliver an influx of haematopoietic cells that give rise to osteoclasts which excavate the cartilage template, and *Osx1*$^+$ osteoblast progenitors and other blood cell types that populate the newly formed marrow cavity. **d** | A longitudinal growth axis is established when vascularization and osteoclast-mediated resorption bisect the presumptive skeletal element, producing two growth plates with opposing directionality. A perpendicular growth axis is driven by periosteal osteoblasts and allows the bone to grow in width. **e** | Within the remodelled cartilage template, bone-forming osteoblasts are derived from *Osx1*$^+$ cells arriving with the invading vasculature, as well as hypertrophic *Col10a1*$^+$ chondroctyes that transdifferentiate as they exit the growth plate into the marrow cavity. As bones grow in length and width, a second wave of vascularization forms the secondary ossification centres. **f** | Mature endochondral bone. Additional information about developmental skeletogenesis and a summary of genetic evidence for involvement of the bone morphogenetic protein pathway in developmental skeletogenesis can be found in the text and in Supplementary information S1–S5 (tables).

As a mechanism to drive growth plate tissue expansion and thereby lengthen the skeletal element, terminally differentiating chondrocytes swell by a multistep process involving a massive increase in fluid and then dry mass[95]. Although some hypertrophic *Col10a1*$^+$ chondrocytes become apoptotic, many exit the growth plate cartilage into the bone marrow space, where they resume the cell cycle and transdifferentiate into osteoblasts. This intriguing phenomenon was first described in chicks[96]; new techniques in lineage-tracing reveal that this chondrocyte-derived pool of osteoprogenitors contributes significantly to osteoblast and osteocyte populations primarily at trabecular sites, but also some endocortical sites[97–100].

Condensation, or compaction, is highly dependent on BMP signalling as it is blocked by the BMP ligand antagonist, gremlin[101]. Accordingly, compensatory activity by ALK2, ALK3 and ALK6 or signalling through SMAD4 in the emerging limb bud is essential for condensation and the earliest steps of chondrocyte differentiation[78,79,102,103]. ALK2 seems to have a more prominent role in the axial skeleton and craniofacial vault as loss of only *Alk2* in early osteochondroprogenitors (*Alk2; Col2A1–Cre*) leads to cranial and vertebral hypoplasia[102]. Curiously, mice with a *Smad4; Col2a1–Cre* mutation exhibit dwarfism as a result of growth plate disorganization, whereas mice with *Smad1; Smad5; Col2A1–Cre* compound mutations develop severe chondrodysplasia, which suggests that at least in chondrocytes, not all BMP effects are mediated by SMAD4 (REFS 104,105). A variety of more modest chondrocyte defects leading to shortened long bones (as found in brachypodism) are found in mice with various conditional genetic manipulations of *Bmp2* or global null deletions of *Gdf5*, *Gdf6* and/or *Bmp5* (REFS 84,85,106–110), again indicating compensatory action by multiple ligands. By contrast, mice with disruption of TGF-β signalling in the *Prx1–Cre* expression domain are able to undergo limb bud chondrogenesis, but develop longitudinal growth defects as chondrocytes differentiate too quickly[111,112]. Impaired vascularization is observed in several of these models[80,81,105,113]; however, it remains unclear whether this effect is secondary due to delay or arrest of chondrocyte hypertrophy. Postnatally, BMP signalling continues to affect growth plate dynamics as loss of *Alk3* in the *Agc1–CreERT2* (REF. 114) expression domain, which

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recombines broadly in differentiating chondrocytes of the perinatal or postnatal growth plate, dramatically arrests longitudinal (but not appositional) growth[115]. In this model, growth plate cartilage is replaced by bone, which suggests that *Alk3* is required for exit of transdifferentiating chondrocytes from the growth plate into the primary ossification centre.

Vascularization closely coincides with formation of a bone collar around the perimeter of the cartilage rudiment. The bone collar is formed by osteoblasts derived from the *Prx1*$^{+}$ progenitors that have transitioned through key checkpoints of osteoblast differentiation marked by sequential expression of *Runx2* (endochondral progenitors), *Osx1* (committed osteoprogenitors), *Col1a1* (differentiating osteoblasts) and *Ocn* (mature osteoblasts). Not only have these molecular markers become useful for tracking cells in the osteogenic lineage, but promoter fragments from each of the genes have now been used to target Cre-mediated recombination to specific subpopulations of osteoblasts[116–118] while sparing recombination in the joint (FIG. 4b). Importantly, some *Osx1*$^{+}$ osteoprogenitors of the perichondrium/bone collar migrate into the cartilage rudiment with invading blood vessels[119] (FIG. 3c), acting together with the osteoprogenitor pool derived from hypertrophic chondrocytes to form the osteoblasts that replace the cartilage template with bone.

Whereas *Bmp7* is dispensable for bone formation (*Bmp7; Prx1–Cre*[83]), co-expression of *Bmp2* and *Bmp4* in the limb bud mesenchyme is essential for osteogenesis (*Bmp2; Bmp4; Prx1–Cre*[80]). Furthermore, several studies reveal that BMP signalling has a fundamental role in formation of a normal bone extracellular matrix. Mice lacking *Smad4* in committed (*Smad4; Osx1–Cre*[120]) or mature (*Smad4; Ocn–Cre*[121]) osteoblasts exhibit dwarfism with small brittle bones that are prone to fracture, resulting at least in part from reduced periosteal growth and a failure to express multiple enzymes required for proper collagen assembly[120]. Loss of *Bmp2* in early endochondral (*Bmp2; 3.6kbCol1A1–Cre*[113]) or committed osteoblast progenitors (*Bmp2; Osx1–Cre*[122]) causes low bone mass and reduced BMD. Teeth are hypomineralized[123,124]; long bones are narrow, brittle and have reduced load to fracture in biomechanical tests[125]. These *in vivo* observations support our recent finding that expression of *Bmp2* is required for *Prx1*$^{+}$ progenitors to make the *Runx2/Osx1*$^{+}$ transition and induce several enzymes required for calcium and phosphate metabolism (V. S. Salazar, unpublished work).

In comparison to the roles for BMP pathway in limb bud outgrowth, chondrogenesis and longitudinal bone growth, a significant amount of mystery remains about the molecular and cellular mechanisms controlling appositional growth (FIG. 3d). This is the only known process by which individual skeletal elements grow in width during early postnatal life. Notably, mice lacking *Bmp2* in early *Prx1*$^{+}$ progenitors develop a severe postnatal phenotype where appendicular bones grow in length but fail to grow in width (L. Capelo and V. S. Salazar, unpublished work). As BMD is also low, bones in these mice exhibit inferior material as well as geometric properties, factors underlying a 100% incidence of spontaneous fractures[81]. *Bmp2; Prx1–Cre* mice are furthermore unable to initiate fracture healing and do not accept bone grafts[81,126]. Similarly, fractures do not heal in mice lacking *Bmp2* in *Col2a1*$^{+}$ early osteoprogenitors[100] (*Bmp2; Col2a1–Cre*[127]). By contrast, mice lacking *Bmp2* in committed osteoprogenitors (*Bmp2; Osx1–Cre*[125]) or differentiated osteoblasts (*Bmp2; 2.3kb-Col1a1–Cre*[127]) exhibit normal fracture healing. Spontaneous fractures are not reported in TGF-β mutant mice, or in mice lacking BMP4 (REF. 82) or BMP7 (REF. 83) in the limb. Appositional bone growth, fracture repair and graft acceptance are all postnatal processes that rely on activation of developmental endochondral ossification programs in the periosteum. Together, these data reveal a unique role for BMP2 in periosteal function during appositional growth and fracture repair, and point to a pre-*Osx1*$^{+}$ cell as a critical source and target of BMP2 in bone.

Upon acquisition of peak adult bone mass (FIG. 3f), BMP signalling affects skeletal homeostasis[128], as mice lacking *Alk3* in mature osteoblasts and osteocytes (*Alk3; OG2–Cre*) develop high bone mass resulting from a state of low bone turnover (cell-autonomous effects on osteoblast activity as well as cell nonautonomous effects on osteoclast-mediated bone resorption)[129]. However, ALK3 is only one of several type I BMP receptors expressed in adult skeletal tissue, and so it remains unclear whether loss of ALK3 alone is sufficient to block all BMP signalling or whether an alternative underlying mechanism to this phenotype is involved. Also of particular interest is a potential role for BMPs in lamellar bone formation or

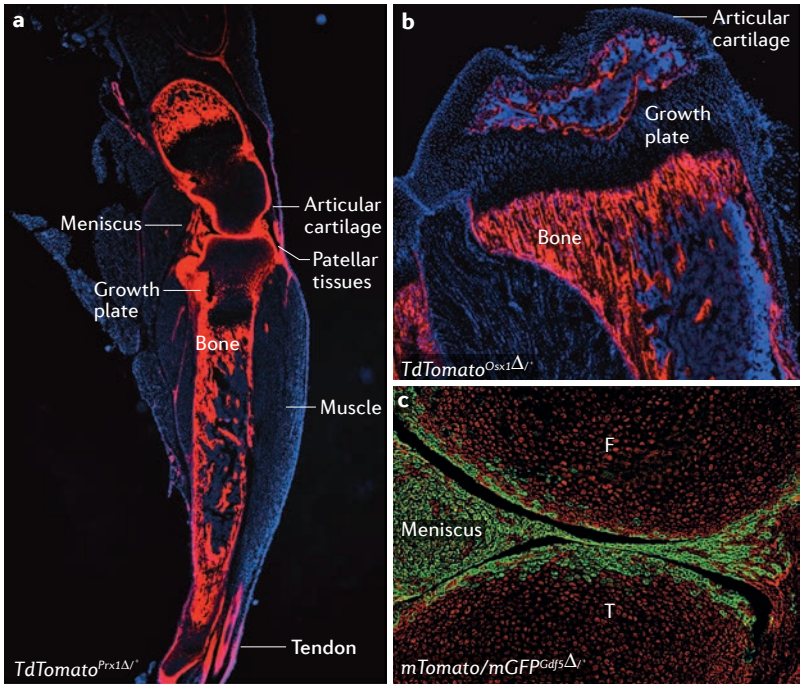

Figure 4 | **Cre-mediated gene recombination in subpopulations of bone cell lineages.** Expression of Tomato and green fluorescent protein (GFP) reporter proteins in hindlimbs of transgenic mice marks specific skeletal populations targeted by different Cre drivers. **a** | *TdTomato*$^{Flox/+}$*; Prx1–Cre* (P0). **b** | *TdTomato*$^{Flox/+}$*; Osx1–Cre* (P14). **c** | *mTomato/mGFP*$^{Flox/+}$*; Gdf5–Cre* (P0). Longitudinal sections of the hindlimb were imaged for red fluorescent protein, GFP and 4′,6-diamidino-2-phenylindole.

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intramembranous fracture healing, such as occurs with mechanical loading or the repair of stress fractures. Data published in 2015 indicate that expression of *Bmp2* in cells of the *Osx1–Cre* lineage is dispensable for these responses in the adult skeleton (*Bmp2; Osx1–Cre*)[125]. It remains unclear whether intramembranous healing and lamellar bone formation require differentiation of new osteoblasts or are mediated instead by activation of existing osteoblasts or bone-lining cells. Although additional studies are required, the initial findings are consistent with a model in which a pre-*Osx1*$^+$ progenitor is a key source and target of the BMP signalling required for osteogenesis. Phenotypes from these and additional studies have been visually summarized for quick reference (FIG. 5) and are presented in a cited catalogue (see Supplementary information S1–S5 (tables)) for additional information.

Joint morphogenesis

Much remains unknown about the molecular and cellular mechanisms by which presumptive endochondral skeletal elements become segmented by joints. The earliest morphological sign of a presumptive joint is the emergence of an interzone (FIG. 6a), a tripartite structure consisting of a mid-density inner layer called the central intermediate lamina, and two high-density outer layers that give rise to the articular cartilage. Although the interzone is initially composed of prechondrogenic *Col2a1*$^+$ cells recruited from within the mesenchymal condensation of the emerging limb bud[130], interzone cells quickly lose their chondrogenic morphological features[131] and downregulate chondrocyte extracellular matrix products, notably *Col2a1* (REF. 130) (FIG. 6b). One of the earliest known molecular markers of interzone specification, *Gdf5,* is induced before interzone condensation[85,107,110,132], most probably by TGF-β signalling, which suggests that TGF-β exerts essential effects at the earliest stages of joint morphogenesis[112,133,134]. Upon initial induction, *Gdf5* expression becomes restricted to a thin strip where neighbouring endochondral skeletal elements undergo segmentation through a still poorly understood process of joint cavitation (FIG. 6c). *Gdf5*$^+$ cells of the interzone give rise to many major cell types and structures of a mature joint, including the tendons, ligaments, synovial membrane, menisci, articular cartilage and zonal enthesis[135–137] (FIG. 6d). *Prx1–Cre* provides a useful tool for targeting gene recombination to cells that comprise the joint (FIG. 4a), and *Gdf5–Cre*[136] for specifically targeting lineages derived from *Gdf5*-expressing cells of the interzone (FIG. 4c).

Although GDF5 is universally recognized as a marker for interzone formation, this factor is also a signalling molecule. GDF5 (also known as CDMP1 or BMP14), GDF6 (also known as CDMP2 or BMP13) and GDF7 (BMP12) represent a subordinated group of BMP ligands[108,110,132]. GDF5, GDF6 and GDF7 share 80–86% sequence similarity with each other, but are more divergent in the mature C-terminal domain compared with other BMPs (~56% similarity with BMP2 and BMP4, 50–54% similarity with BMP5, BMP6, BMP7 and BMP8 and 46–47% similarity with BMP3)[110]. By mechanisms similar to BMPs, GDF5, GDF6 and GDF7 can activate BMP receptors and SMAD1/5/8 signalling, and can be sequestered by the secreted BMP pathway antagonist noggin[138]. GDF5 binds to all three BMP type II receptors (ACVR2A, ACVR2B and BMPR2), but exhibits highly preferential binding and signalling via the type I receptor BMPR1B. GDF5 does not signal through BMPR1A[139–141]. *Gdf5*, *Gdf6*, *Gdf7*, *Bmp2*, *Bmp4* and *Nog* mRNAs are each reported to be expressed at the interzone[84,85,132]. These observations led to the hypothesis that the BMP pathway has a role in joint morphogenesis. Consistent with this idea, mice globally lacking *Nog* (which encodes noggin) exhibit a block in joint morphogenesis and widespread shortening of endochondral bone and cartilage structures[58]. In addition, heterozygous mutations in *NOG* were subsequently identified in patients as the underlying cause of proximal symphalangism (SYM1) and type I multiple synostoses (SYNS1)[142], genetic disorders of joint morphogenesis. Curiously, mice lacking *Bmp2*, *Bmp4* or *Bmp7* in *Prx1–Cre* expressing cells of the developing limb are able to form joints normally[81–83]. These findings suggest that GDFs provide the specialized BMP function required for joint morphogenesis. In particular, *Gdf5* is expressed with great specificity at developing interzones throughout the skeleton. Despite its widespread interzone expression pattern, mice and humans lacking GDF5 display defects in joint morphogenesis in only a subset of synovial joints, most notably those of the wrists and ankles. These same joints are also abnormal when too much GDF5 activity is present[143]. As a group, *Gdf5, Gdf6* and *Gdf7* have partially overlapping mRNA expression patterns at distinct joint sites, which suggests there might be compensation for loss of any individual *Gdf* and, in fact, mice with compound deficiencies in *Gdf5, Gdf6* and *Gdf7* exhibit synostoses in a greater number of joints relative to those with single deficiencies[84]. However, the phenotype of *Gdf5*; *Gdf6*; *Gdf7* compound mutant mice does not recapitulate the phenotype of mice with *Nog* deficiency, particularly in large joints, including the hips and knees.

Thus, although roles for noggin, GDF5, GDF6 and GDF7 in joint morphogenesis are evident, much remains unknown about the mechanism by which they induce joint formation. This lack of knowledge arises in large part from the fact that noggin is a BMP antagonist, whereas GDF5, GDF6 and GDF7 are considered by classic models to be BMP agonists, which presents a challenge to understanding whether BMP signalling must be active or repressed at the presumptive joint site for joint morphogenesis to progress properly. One intriguing possibility is that BMP signalling activity must be restricted at inner layers of the interzone to suppress chondrogenesis where the joint needs to cavitate, but be active in the outermost edges of the interzone where an articular surface must be specified and mature. Several observations support this model, and furthermore suggest that this signalling pattern is accomplished by differential expression domains of type I BMP receptors. During joint morphogenesis, *Gdf5* and *Bmpr1a* are highly co-expressed at the interzone where

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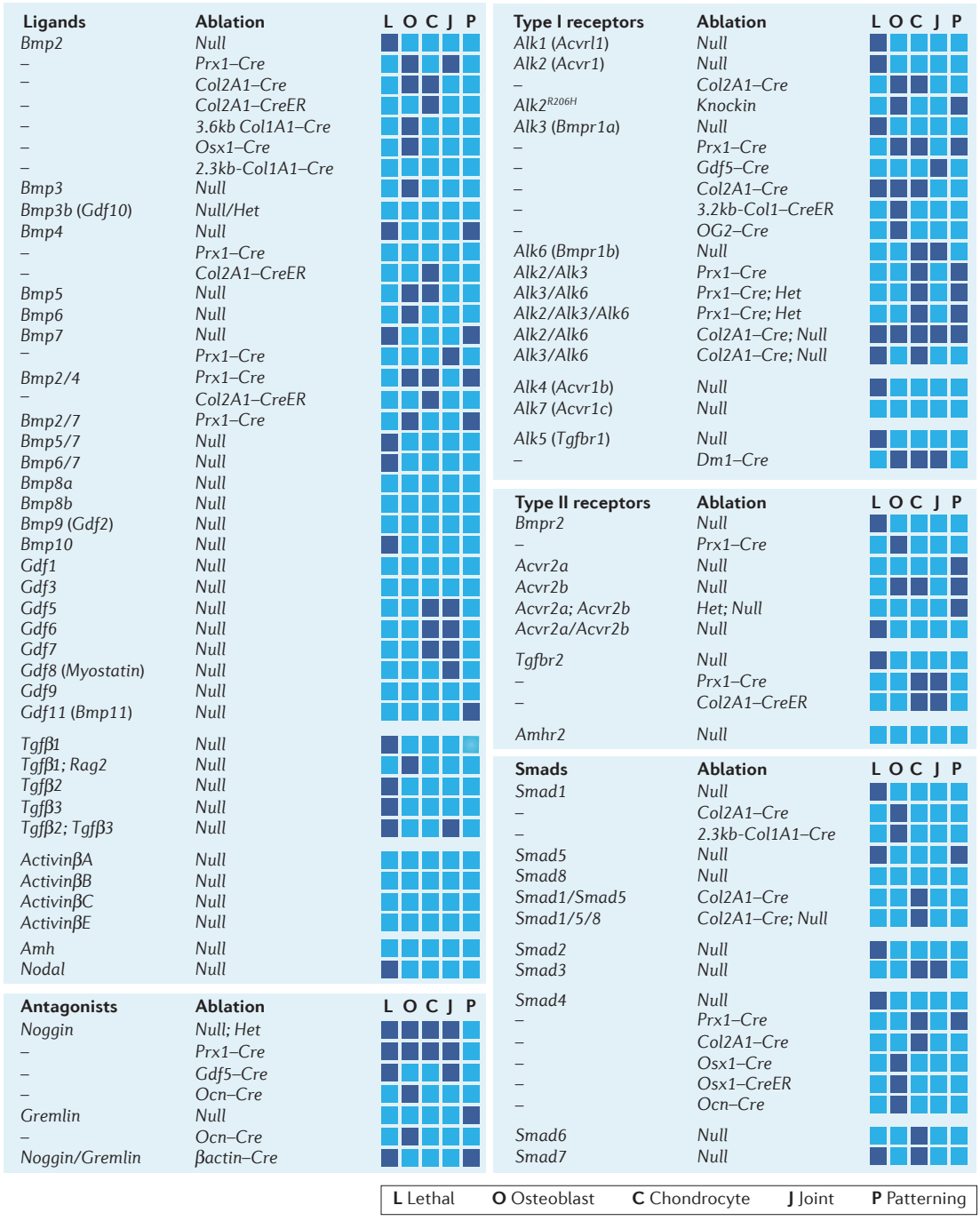

Figure 5 | **Reported contributions by BMP pathway to skeletal biology.** Summary of skeletal phenotypes observed in experimental mouse models where genes encoding components of the bone morphogenetic protein (BMP) superfamily pathway have been disrupted using global or conditional gene targeting. Models include nulls, conditional knockouts or gene replacements of endogenous loci, but not transgenics. Osteoblast defects include problems with developmental ossification, postnatal skeletal overgrowth at nonskeletal sites, periosteal growth, altered amount of bone mass and an abnormal quality of bone matrix including brittleness, spontaneous fractures, disrupted fracture repair, scoliosis and kyphosis. Cell non-autonomous effects on osteoclasts are not included. Chondrocyte defects consist of chondrodysplasia, dwarfism, longitudinal growth defects (including the short bone phenotype component of brachypodism), impaired or accelerated chondrogenesis and defects in vascularization of the cartilage template. Joint defects encompass failure to form synovial or nonsynovial joints during development, problems generating mature joint structures such as the meniscus or tendons and/or ligaments and osteoarthritis. Patterning defects include failed outgrowth of the limb bud, vertebral transformation, craniofacial malformation, bone deformities (size), altered number of digits, and lateral fusions of perichondrium in zeugopod. Dark blue squares represent positive for model; turquoise squares represent negative for model. A comprehensive and cited catalogue of these and additional mouse models can be found in Supplementary information S1–S5 (tables), human disease associations in TABLE 1.

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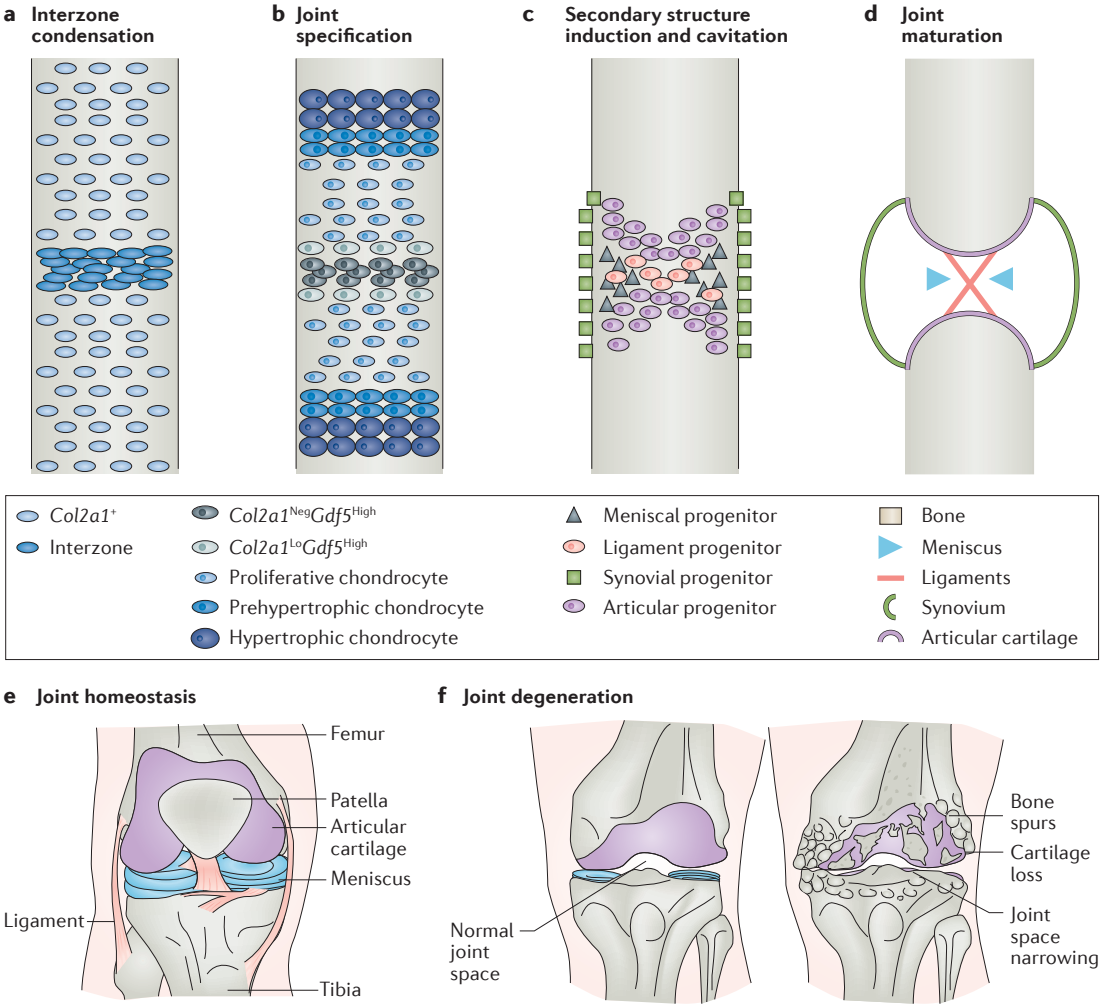

Figure 6 | **Joint morphogenesis.** Longitudinal views depicting key steps in the formation of the knee joint. **a** | The first sign of a presumptive joint is a condensation of *Col2*$^+$ limb bud progenitors at the presumptive joint site. **b** | Joint specification is marked by induction of *Gdf5* in the interzone and downregulation of *Col2a1*. **c** | A joint space is formed by cavitation after progenitors for a variety of secondary joint structures are specified from the *Gdf5*$^+$ progenitor pool. **d** | Maturation of the synovial joint of the knee occurs during development and early postnatal life. **e** | Schematic representation of a healthy human knee. **f** | Joint health in adult life is affected by genetics and environmental factors such as nutrition and exercise. Loss of joint homeostasis can trigger degenerative joint diseases such as osteoarthritis, which is characterized by degradation of articular and meniscal cartilage, formation of bone spurs and pain. Additional information about joint morphogenesis and a summary of genetic evidence for involvement of the bone morphogenetic protein pathway in joint morphogenesis can be found in the text and in Supplementary information S1–S5 (tables).

segmentation takes place[109]. By contrast, *Bmpr1a* and *Bmpr1b* are co-enriched in regions flanking the interzone, where articular chondrocyte progenitors are specified and differentiate[144,145]. Only after cavitation does expression of *Bmpr1a*, *Bmpr1b* and *Gdf5* become co-restricted to the articular surface[145]. Thus, although *Gdf5* expression significantly overlaps with that of *Bmpr1a* in the early interzone, it does not seem to significantly overlap with *Bmpr1b* until joint cavitation and specification of articular prechondrocytes. Signalling of GDF5 through BMPR1B, but not BMPR1A, suggests that activating BMP signalling might be a critical function for GDF5 during articular cartilage formation but is not the function for GDF5 deep within the interzone. Accordingly, mice lacking *Bmpr1a* in the *Gdf5–Cre* expression domain can form most joints but do not establish a mature articular cartilage[137]. Also consistent with a model where GDF5 does not activate BMP signalling in the interzone is the prototypic regression of interzone cells from a *Col2A1*$^+$ cell phenotype, where *Col2A1* expression is an established hallmark of limb bud progenitors engaged in BMP signalling[146]. A role for ALK2 in joint morphogenesis requires further investigation, but is likely to unfold in the axial skeleton where deficiency of *Alk2* in early osteochondroprogenitors (*Alk2; Col2A1–Cre*) causes segmentation defects[102], and activated alleles of *ALK2*, such as those found in patients with fibrodysplasia ossificans progressiva, produce congenital malformations at costovertebral joints and fusions at cervical sites[74].

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Multiple studies also demonstrate that TGF-β signalling molecules are expressed at the developing joint and are required for induction of *Gdf5*, *Nog* and *Jagged-1* mRNAs at the interzone, which suggests that TGF-β exerts essential effects at the earliest stages of joint morphogenesis[112,133,134]. Although germline deletion of *Alk5* (*Tgfbr1*) or *Tgfbr2* is embryonic lethal[147,148], *Tgfbr2; Col2–Cre* mice exhibit joint defects, mostly at costal and vertebral sites, with loss of intervertebral disks[149]. Mice lacking *Tgfbr2* in the *Prx1–Cre* expression domain fail to form a *Gdf5*$^{+}$ interzone in the digits, and missing phalangeal joints are replaced by expanded regions of cartilage[111,112,134]. Tendons and ligaments are also lacking. This phenotype is recapitulated by compound loss of *Tgfb2* and *Tgfb3*, and provides a possible rationale for patellar aplasia in *Tgfbr2*; *Prx1–Cre* mice, as the patella is a sesamoid bone that forms within the patellar tendon[150]. After joint formation, *Tgfbr2* is highly expressed in the synovio-entheseal complex that includes mature joint structures such as the synovium, tendon entheses, articular cartilage and perichondrium[133]. Phenotypes from these and additional studies have been visually summarized for quick reference (FIG. 5) and presented in a cited catalogue (see Supplementary information S1–S5 (tables)) for additional information.

Skeletal disorders

Altered BMP superfamily signalling

Skeletal dysplasias are a large heterogeneous collection of human diseases typified by abnormal formation and growth of bones, joints or connective tissues. Although collectively rare, the prevalence of all osteochondrodysplasias is estimated to be 7.5 per 10,000 during pregnancy and 2.4 per 10,000 at birth[151,152]. At least 436 distinct disorders have been identified thus far, resulting from mutations in 364 genes[153]. Consistent with experimental data in mice supporting a key role for BMP superfamily signalling in skeletal development, mutations in genetic loci encoding BMP pathway molecules are the cause of a variety of skeletal disorders in humans (TABLE 1). Although certain groups of skeletal disorders seem to share a core set of overlapping features, they have by historical convention been subdivided and given unique names according to criteria such as differing modes of inheritance or relationships to distinct genetic loci. In the advent of genomics and other forms of big-data science, it has become increasingly difficult to navigate this classic system of disease nomenclature as >138 new genes were linked to human skeletal dysplasias between 2011 and 2015, representing an ~61% increase in just 4 years in the number of loci attributable to disorders of the skeleton[153]. More importantly, however, this advance provides opportunities to form testable hypotheses about genotype–phenotype correlations within groups of disorders that are phenotypically similar but genetically distinct, which might be particularly useful for understanding diseases with recognizable features but with unknown aetiology. As a strategy to handle this increasing complexity, and with the idea that clinical overlap can often be explained by commonalities in underlying molecular mechanisms, we next discuss several groups of phenotypically similar but genetically distinct skeletal disorders and hypotheses about whether an excess or deficiency of signalling through specific branches of BMP–GDF or TGF-β–activin signalling pathways is involved.

Altered TGF-β signalling. Skeletal overgrowth is a defining feature of Loeys–Dietz syndrome (LDS)[154], a highly variable disease affecting connective tissues. Five subtypes of LDS represent distinct molecular aetiologies of altered TGF-β signalling[8,155]. LDS1 arises from autosomal dominant mutations in *TGFBR1* (MIM 609192, chromosome 9q22.33). LDS2 is linked to *TGFBR2* (MIM 610168, chromosome 3p24.1). LDS3 arises from mutations in *SMAD3* (MIM 613795, chromosome 15q22.33). LDS4 and LDS5 are driven by mutations in *TGFB2* (MIM 614816, chromosome 1q41) and *TGFB3* (MIM 615582, chromosome 14q24.3), respectively. The mechanism remains difficult to understand as most mutations identified so far should disrupt signalling but instead seem to be associated with excessive TGF-β signalling. Affected patients have craniosynostoses, scoliosis, a sunken or bulging chest, club or flat feet, and contractures of the joints. Many patients exhibit degeneration of intervertebral discs, osteoarthritis and frequent joint dislocations, are highly susceptible to vascular complications including aortic aneurysm, and skin appears transluscent, develops stretch marks and bruises, and forms abnormal scars. Immune-system dysfunction and inflammatory disorders are common. Camurati–Engelmann disease (CED, MIM 131300) is a rare autosomal dominant sclerosing bone disorder linked to *TGFB1* (chromosome 19q13.1)[156–158]. CED mutations are speculated to increase TGF-β signalling through mutations such as Arg218Cys, which affects secretion and activity of TGF-β. Patients exhibit increased cortical thickness in the limbs and skull, which leads to neurological problems owing to increasing pressure on the brain. Scoliosis, joint contractures, knock knees and flat feet are frequently reported[156–158].

Notably, the limbs of patients with LDS or CED are disproportionately long compared with their height. Long limbs are a prototypic feature of Marfan syndrome, a disease of increased TGF-β signalling resulting from mutations in *FBN1* that prevent TGF-β from being stored properly in the skeletal extracellular matrix. Patients with CED also have considerable muscle atrophy, consistent with data showing that increased bioavailability of TGF-β caused by osteolytic bone metastasis can trigger oxidation of skeletal muscle[159]. Thus, muscle weakness and long limbs are symptoms consistent with excess TGF-β signalling. What is not consistent about this genotype–phenotype correlation is extensive evidence that TGF-β is a negative regulator of bone mass. As systemic blockade of TGF-β by agents such as the 1D11 antibody (a neutralizing antibody that recognizes TGF-β1, TGF-β2 and TGF-β3) improves bone mass in mice with Marfan syndrome[160], osteogenesis imperfecta[161] or osteolytic bone metastases[159], the mechanism for sclerosis in CED is unclear.

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Table 1 | **Human disorders associated with mutations in genes encoding components of the BMP pathway**

Gene	Mutation	Human disease associations
Ligands		
BMP2	Duplication, 3′ regulatory region	Brachydactyly type A2 (MIM 112600)
BMP4	LOF	Oralfacial cleft 11 (MIM 600625); microthalmia 6 (MIM 607932)
BMP9 (*GDF2*)	LOF	Hereditary haemorrhagic telangiesctasia, type 5 (MIM 615506)
BMP15	LOF	Ovarian dysgenesis 2 (MIM 300510)
GDF1	LOF	Cardiac defects (MIM 217095, 208530, 613854)
GDF3	Missense	Klippel–Feil syndrome 3 (MIM 613702); microthalmia (MIM 613703, 613704)
GDF5	SNPs in 5′ UTR, LOF, haplo-insufficiencies, GOF	Osteoarthritis (MIM 612400); Brachydactyly, type A1 (MIM 615072), type A2 (MIM 112600), type C (113100); chondrodysplasia, Grebe type (MIM 200700); acromesomelic dysplasia, Hunter–Thompson type (201250); fibular hypoplasia and complex brachydactyly, Du Pan syndrome (228900); multiple synostoses syndrome 2 (MIM 610017); proximal symphalangism, 1B (MIM 615298)
GDF6	Missense	Klippel–Feil syndrome 1 (MIM 118100); Leber congenital amaurosis 17 (MIM 615360); microthalmia (MIM 613703, 613094)
GDF8 (*MSTN*)	Missense	Muscle hypertrophy (MIM 614160)
TGFB1	GOF	Camurati–Engelmann disease (MIM 131300); modifier of cystic fibrosis (MIM 219700)
TGFB2	LOF	Loeys–Dietz syndrome, type 4 (MIM 614816)
TGFB3	GOF; LOF	Loeys–Dietz syndrome type 5 (MIM 615582); arrhythmogenic right ventricular dysplasia 1 (MIM 107970)
AMH (*MIS*)	LOF	Persistant Mullerian duct syndrome, type I (MIM 261550)
Type I receptors		
ALK1 (*ACVRL1*)	LOF	Hereditary haemorrhagic telangiesctasia, type 2 (MIM 600376)
ALK2 (*ACVR1*)	Arg206His	Fibrodysplasia ossificans progressiva (MIM 135100)
ALK3 (*BMPR1A*)	Heterozygous LOF	Juvenile polyposis syndrome (MIM 174900); polyposis syndrome, hereditary mixed 2 (MIM 610069)
ALK4 (*ACVR1B*)	LOH	Pancreatic cancer, somatic (MIM in progress)
ALK5 (*TGFBR1*)	Missense	Loeys–Dietz syndrome, type 1 (MIM 609192)
ALK6 (*BMPR1B*)	Heterozygous LOF	Brachydactyly, type A2 (MIM 112600); acromesomelic dysplasia with genital anomalies, Demirhan type (MIM 609441)
Type II receptors		
BMPR2	Heterozygous LOF	Familial pulmonary arterial hypertension (MIM 178600); pulmonary venoocclusive disease 1 (MIM 265450)
ACVR2B	Missense	Heterotaxy, visceral, 4, left-right axis defects (MIM 613751)
TGFBR2	Missense	Loeys–Dietz syndrome, type 2 (MIM 610168); colorectal cancer, hereditary nonpolyposis, type 6 (MIM 614331); oesophageal cancer, somatic (MIM 133239);
AMHR2 (*MISR2*)		Persistant Mullerian duct syndrome, type II (MIM 261550)
SMADs		
SMAD3	Missense	Loeys–Dietz syndrome, type 3 (MIM 613795)
SMAD4 (*DPC4*)	LOF/LOH	Myhre syndrome (MIM 139210); juvenile polyposis/hereditary haemorrhagic telangiectasia syndrome (MIM 175050); Pancreatic cancer, somatic (MIM 260350); polyposis, juvenile (MIM 174900)
SMAD6	Missense	Aortic valve disease (MIM 614823)
SMAD8 (*SMAD9*)	Nonsense	Pulmonary hypertension (MIM 615342)
Antagonists		
LEFTY2	Human mutations	Left-right axis malformations (MIM in progress)
NOGGIN	Missense	Brachydactyly, type B2 (MIM 611377); multiple synostoses syndrome 1 (MIM 186500); Stapes ankylosis with broad thumb and toes (MIM 184460); symphalangism, proximal (MIM 185800); tarsal-carpal coalition syndrome (MIM 186570)

Human genetic disorders described are restricted to conditions that have achieved peer-reviewed status with a MIM number. BMP, bone morphogenetic protein; GOF, gain of function; LOF, loss of function; LOH, loss of heterozygosity; MIM, Mendelian inheritance in man; SNP, single nucleotide polymorphism; UTR, untranslated region.

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Reduced BMP signalling. Acromelic dysplasias, acromesomelic dysplasias and brachydactylies are a group of human diseases characterized, among other distinguishing features, by severe longitudinal growth defects that manifest at distal sites of the limb while skeletal structures of the skull and spine are reasonably normal. Patients with these skeletal disorders have variable types of dwarfism and other limb deformities resulting from genetic perturbations to the loci encoding the ligand GDF5 (*GDF5*, chromosome 20q11.22)[162], its secreted antagonist Noggin (*NOG*, chromosome 17q22), its high affinity receptor BMPR1B (*BMPR1B*, chromosome 4q22.3), or the downstream signalling mediator SMAD4 (*DPC4*, chromosome 18q21.1). Acromesomelic dysplasia Du Pan type (AMD, MIM 228900) is the most mild in clinical features and is linked primarily to missense mutations in *GDF5* (such as Leu441Pro) that disrupt the ability of GDF5 and BMPR1B to form a signalling complex[163,164]. Patients with Du Pan exhibit typical features of distal limb brachydactyly (short fingers and toes) alongside fibular aplasia[165,166]. Dislocations of the knee, patella or ankle are often reported[166]. AMD Hunter type (AMDH, MIM 201250)[167,168] is also linked to mutations in *GDF5*, but with severe brachydactyly in the hands, feet, tibia and humerus. Joint dislocations are common. AMD Grebe type (AMDG, MIM 200700) has been attributed to mutations that reduce secretion of GDF5 (REF. 169), as well as missense and nonsense recessive mutations of *BMPR1B*[170]. Grebe chondrodysplasia is more severe than Hunter or Du Pan types, with extreme short-limb dwarfism, loss of carpal and/or tarsal articulations, absence of proximal and middle phalanges and some metacarpals, and occasionally polydactyly in the hand; residual structures of fingers and toes appear as skin appendages[171]. A gene dosage effect seems to be operative in a Grebe pedigree as patients who are heterozygous have normal height with mild brachydatyly, postaxial polydactyly, or flexion and/or contraction of fingers[171]. Mutations in *BMPR1B* also cause AMD Demirhan type (AMDD, MIM 609441), in which severe limb formations are comprised of brachydactyly, ulnar deviation of the hands, fusion of the carpal and/or tarsal bones, fibular hypoplasia and/or aplasia, and even club-foot[172]. As a defining feature, patients with AMDD also have reproductive anomalies such as absence of ovaries, hypoplasia of the uterus and primary amenorrhoea.

In the brachydactyly group, types A2, B2 and C are most typically diagnosed without extraskeletal malformations or genital anomalies. Brachydactyly type A2 (BDA2, MIM 112600) is a chondrodysplasia affecting the middle phalanges of the second and fifth fingers. BDA2 is similar to Grebe[170] in both clinical presentation and molecular aetiology, although with autosomal dominant mutations in the BMPR1B–GDF5–BMP2 signalling axis. Several alleles associated with BDA2 have been identified that reduce binding affinity between GDF5 and BMPR1B proteins[162,173–175]. Brachydactyly type C (BDC, MIM 113100) is also linked to autosomal dominant mutations in *GDF5*, which can be insertions or deletions that lead to frameshifts or early termination[176–178]. BDC has substantial clinical variability and can skip generations, which suggests that genetic modifiers of the disease exist[179].

The last member in this group is an acromelic dysplasia known as Myhre syndrome (MYHRS, MIM 139210)[180,181]. Approximately 30 cases of MYHRS have been documented so far and all are linked to somatic autosomal dominant mutations in *SMAD4* (also known as *DPC4*, 18q21.1) that are proposed to reduce the activity of SMAD4 (REF. 182). In addition to brachydactyly, there is microcephaly, mental retardation, small eyes and a constellation of skeletal features such as a protruding jaw, short stature, conductive hearing loss, a thick skull, flat vertebrae, broad ribs, hypoplastic iliac wings and stiff joints with limited mobility. Patients with MYHRS can also exhibit an unusually muscular build, age-associated cardiac and pulmonary defects and abnormal wound healing. Thus, MYRHS combines some key features associated with disrupting mutations in BMP–GDF signalling (brachydactylies and growth retardation) with features more commonly associated with disrupting mutations in TGF-β–activin–GDF8 pathways (muscle hypertrophy, joint mobility issues, abnormal wound healing and cardiac and/or pulmonary defects). This combination of features is reasonable to expect as SMAD4 interacts with both branches of R-SMADS and thus represents a common node used by both BMP/GDF and TGF-β/activin pathways to transduce signals.

Acromelic dysplasias, acromesomelic dysplasias and brachydactylies, therefore, have partially overlapping features characterized by severe longtitudinal growth defects in the limbs. Clinically, they are distinguished by the specific skeletal sites affected by disease and the underlying genetic locus affected by the mutation, as well as by autosomal dominant versus recessive modes of inheritance. However, from a molecular perspective, accumulating data strongly suggest that these disorders share a common underlying molecular mechanism, which consists of reduced signalling through the GDF5–BMPR1B–SMAD4 signalling axis. And indeed, diminished BMP signalling as a cause of human skeletal growth defects strongly correlates with experimental evidence obtained in mice where *Gdf5*, *Bmpr1B* and *Smad4* mediate BMP signals in growth plates of endochondral bones to drive longitudinal bone growth[84,85,94,104–110,183]. The number of affected skeletal sites and overall severity of brachydactyly increases when underlying mutations are autosomal dominant or target downstream signalling molecules such as BMPR1B or SMAD4. Brachydactylies of unknown aetiology might be tested in a hypothesis-based manner for loss-of-function mutations in genes such as *GDF6*, *GDF7*, *SMAD1* or *SMAD5*, which can also contribute to this pathway in skeletal tissues. One interesting unresolved question concerns how heterozygous mutations in *GDF5* exert dominant effects on the signalling pathway. Possibly, these mutant proteins can dimerize with wild-type GDF5 and GDF6, which results in the formation of both homodimers and heterodimers with diminished binding affinity for BMPR1B.

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Excess BMP signalling. Part of the persisting challenge in understanding the molecular mechanisms driving various types of brachydactylies arises from the fact that altered BMP signalling can inhibit longitudinal growth by at least two cellular mechanisms: impaired chondrogenesis due to inadequate BMP signalling, and accelerated chondrogenesis due to excessive BMP signalling. A potential example of the latter might be BDA2, which results from a 5.5 kb microduplication of a highly conserved but noncoding sequence 110 kb downstream of *BMP2* (chromosome 20p12.3)[184,185]. Both the placement and conservation of this duplication suggest that it contains a distal enhancer that controls the expression of *BMP2* in regions where GDF5–BMPR1B signalling is also active; a LacZ reporter construct was found to be highly expressed in developing mouse skeletal tissues when placed under the transcriptional control of this duplicated human genomic fragment[185]. *BMP2* haploinsufficiency does not cause brachydactyly, so this duplication is more likely to attenuate growth by enhancing *BMP2* expression and accelerating chondrogenesis.

Another example might be Brachydactyly type B2 (BDB2, MIM 611377). Patients with BDB2 lack terminal structures of the digits and toes due to missense mutations in *NOG* (chromosome 17q22) that disrupt the antagonist's ability to sequester GDFs and BMPs[186]. These mutations are often referred to as loss-of-function mutations despite the fact that there is increased BMP signalling and, thus, probably an acceleration in chondrocyte differentiation. Features of BDB2 diverge in some ways from classic brachydactylies in that they are often accompanied by soft tissue syndactylies and symphalangism of proximal interphalangeal joints (SYM1, MIM 185800), which correlates well with mouse models in which deficiency of *Nog* produces severe defects in both longitudinal growth and joint morphogenesis[58].

Joint morphogenesis defects are a defining feature of a variety of human symphalangisms, synostoses and dysostoses. Proximal symphalangism type 1 (SYM1A, MIM 185800) is an autosomal dominant disorder consisting of joint defects at proximal interphalangeal, carpal and tarsal sites, often accompanied by conductive hearing loss. A variety of mutations in *NOG* that cause SYM1A have been identified with distinct molecular consequences, including inability of the antagonist to dimerize[187], or loss of heparin-binding activity, which disrupts the ability of noggin to sequester BMPs in the extracellular matrix[188]. Autosomal dominant mutations in *NOG* cause multiple synostoses syndrome 1 (SYNS1, MIM 186500)[189], in which patients have multiple joint fusions, particularly in the hands, conductive hearing loss, a broad nose, thin upper vermillion, radial dislocations and brachydactyly. Autosomal dominant mutations in *NOG* also cause Stapes ankylosis with broad thumb and toes, no symphalangism (SABTT, 184460)[190], in which patients have hearing loss due to fusion of bones in the ear, hyperopia, broad thumb and first toe, but lack evidence of carpal and/or tarsal fusions or symphalangism. Multiple synostoses syndrome 2 (SYNS2, MIM 610017)[191] is similar to SYNS1 but can also include vertebral fusions. Proximal symphalangism type 1B (SYM1B, MIM 615298) is also linked to mutations in GDF5 that increase BMP signalling[173]. Whereas joint morphogenesis defects associated with GDF5 or BMPR1B tend to manifest in the appendicular skeleton, dominant mutations in *GDF6* and *GDF3* cause Klippel–Feil anomaly with laryngeal malformation, a vertebral dysostoses with or without costal involvement (*GDF6*; MIM 148900 and *GDF3*; MIM 613702)[192]. The mechanism behind Klippel–Feil is less clear, but might involve excess BMP signalling through ALK2 as this receptor contributes to axial skeletal development in mice[102]. Notably, GDF3 binds to ACVR2A and ACVR2B, which are shared receptors for BMP ligands. As GDF3 is a weak agonist of activin-type signalling, occupancy of ACVR2A and/or ACVR2B by wild-type GDF3 can reduce the number of type II receptors available for BMPs, which, in turn, attenuates BMP-like signals without greatly enhancing activin-like signals[193]. As both BMPs and BMP type I receptors are abundantly expressed in axial skeletal tissues, mutations that reduce GDF3 expression or GDF3 binding affinity for ACVR2A and/or ACVR2B could lead to excess BMP signalling — an environment that favours bone and cartilage formation at the expense of the formation of joint structures.

Fibrodysplasia ossificans progressiva (FOP, MIM 135100)[194], one of the most rare but disabling skeletal diseases known, develops when the body's repair mechanism goes awry, which causes muscles, tendons and ligaments to ossify when damaged. FOP typically presents in early childhood with extraskeletal ossification starting in the neck and shoulder, although malformations of the big toe are often evident at birth and help to distinguish FOP from other skeletal disorders. Flare-ups are episodic but the crippling accumulation of bone at extraskeletal sites is permanent, which leads to early lethality[195]. FOP is caused by missense mutations in *ALK2* (*ACVR1*), most notably Arg206His[6], that alter the tertiary structure of the BMP receptor in such a way as to confer acquired activation potential of SMAD1–SMAD5–SMAD8 signalling by activins[55]. Systemic blockade of activins has been shown to ameliorate cancer-induced cachexia, which raises the possibility that similar agents might be used to control excess BMP signalling caused by activins in patients with FOP[196].

Therapeutic potential of the BMP pathway

Bone repair

Of all the tissue types in the skeletal system, bone has the most exceptional intrinsic capacity for repair. Evidence that human fractures were manually set, or even surgically treated, can be found in human skeletal remains from the time of Neanderthals[197], ancient Egytians[198], Hippocrates[199] and the Iron Age[200]. Fractures previously healed by endochondral ossification can be easily identified by the presence of a fracture callous or scar. Remarkably, fracture healing and formation of a fracture callous is not restricted to mammalian vertebrates, but rather is clearly documented in diverse species throughout the osteoarcheological record including reptiles from the Paleozoic period and Jurassic theropod dinosaurs[201]. Fracture repair is thus a highly conserved biological process, which suggests that a core set of cell types and signalling molecules required for bone development and repair arose in the

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earliest skeletogenic 'tool kits'. Although most fractures heal without intervention, ~10% result in non-union[202], which greatly increases patient morbidity due to infection and increased hospital stay. BMP therapy has shown considerable success in the healing of recalcitrant fractures, which is consistent with evidence in mice that periosteal BMP2 is required for fracture repair[36]. BMP2 and BMP7 have been approved as adjunct therapies for the treatment of non-union fractures, where the benefits of treatment include accelerated healing and lower infection rates. Clinical data, as well as potential concerns relating to the dose of BMP required, the mode of delivery of BMPs and the cost of treatment has been reviewed elsewhere[203].

At present, clinical use of BMPs is best characterized in procedures that require bone grafts. Estimates indicate that >500,000 bone grafting surgeries are performed every year in the USA, brought about by the need to repair or replace skeletal defects caused by trauma, tumour resection, pathological degeneration and congenital malformation[204]. Autografts, or bone harvested from the patient's own skeleton, are the first choice for successful bone repair, but these are of limited supply and, for many patients, the additional trauma necessitated by graft harvest and the subsequent recovery of the graft site are significant contraindications. Allografts, or bone harvested from cadavers, are more readily available and provide structural support similar to native bone. However, as allografts are devoid of skeletal stem cells and osteoinductive factors, graft incorporation is driven solely by host bone, which can be a slow process, especially in medically compromised and elderly patients. Accumulation of microdamage and fatigue weakening within the allograft occur during the biological replacement process, which leads to a failure rate of 20–25% in the first 5 years after surgery, and ~60% at 10 years after surgery[204]. Mouse models of bone engraftment have identified BMP2 as the stimulus required by both host and graft periosteal cells to initiate the repair response during grafting procedures, and loss of BMP2 production by either graft or host periosteal cells results in the absence of callus formation[205–207]. Moreover, enhancing local BMP2 availability promotes allograft healing via maximal new bone formation while decreasing the potential for fibrosis at the host–graft interface, which is another important clinical concern[126,207–209]. BMP2 and BMP7 are commercially available for clinical use during spine-fusion surgery in place of bone grafts, and have shown efficacy equal to that of using autograft for establishing bone union[5,210,211]. A large body of literature exists that details the success of BMPs in forming new bone, and also concerns relating to the dose of BMP required for healing, the mode of BMP delivery and the potential for unwanted heterotopic ossification at neighbouring sites. A comprehensive review of available clinical data can be found elsewhere[212,213].

Joint repair

Accumulating evidence strongly points to common joint traumas, such as those acquired during sport or overuse injuries, as a key factor underlying development of degenerative arthritis later in life, although genetics also has a large part in disease progression. By 85 years of age, nearly 1 in 4 people will have osteoarthritis of the hip, and 1 in 2 will have osteoarthritis of the knee[214], costing an estimated US $80 billion per year in health-care-related expenses[215]. Current therapies using cell or tissue grafts to repair articular cartilage and other connective tissues in joints have met with limited success, perhaps in part due to the fact that the signalling and transcriptional mechanisms governing the induction of joints and the specification of joint-derived cell types remain largely uncharacterized. Another confounding issue is that most studies of joint morphogenesis have investigated small joints such as the wrists, ankles and those in the digits, whereas joint traumas and diseases requiring medical intervention typically affect larger, more complex joints such as the hip and knee. As larger joints contain distinct structures, have distinct cell proliferation pathways and exhibit unique gene expression profiles during development[216], understanding the developmental signature of large joints will be critical to discovering how joint repair and regeneration can be activated in these joints in adult patients.

Controlling BMP superfamily signalling has emerged as a potential method for inducing stem cells to repair joint tissues such as articular cartilage[217,218]. To begin addressing the role for BMP signalling in joint formation and repair, we are currently utilizing mice expressing *Gfp* under the control of a BMP responsive promoter element (*BRE-Gfp*)[219] to profile when and where BMP signalling is active in skeletal tissues. Preliminary data has already revealed that, at least at birth, BMP signalling is not consistent across all joints, such as the knee and elbow (FIG. 7), supporting our belief that more studies are needed on the specific joints targeted by trauma or disease in patients. These data also raise the possibility that BMP signalling must be temporally and spatially dynamic, so as to differentially accommodate for developmental (FIGS 6a–d) versus homeostatic processes (FIG. 6e) in each joint. Certainly, joint homeostasis itself might be particularly sensitive to optimal signalling thresholds as BMP signalling has been reported to be both necessary to maintain the knee joint[137,220] and associated with the development of osteoarthritis of the knee in mice[9,10] (FIG. 6f). As mentioned previously, GDF5 can deliver BMP-like signals and, in fact, genome-wide association studies have consistently identified noncoding variants in the *GDF5* locus as enhanced risk indicators for osteoarthritis of the knee, hip and wrist[221–223]. As GDF5 is both a decisive marker of joint morphogenesis and a regulatory factor for homeostasis of adult joints, discovering how *Gdf5*$^{+}$ interzone cells are specified during development and maintained in adult life represents a major step forward in determining ways to induce repair or regeneration in a multitude of structures within adult joints.

Conclusions

For much of human history, the skeleton has been regarded as a static organ providing structure, locomotion and protection for soft tissues of the body. It is now widely appreciated that in addition to its role in movement and structure, bone is actually a highly dynamic living organ that contributes, in large part, to haematopoiesis, mineral homeostasis and endocrine control of energy metabolism.

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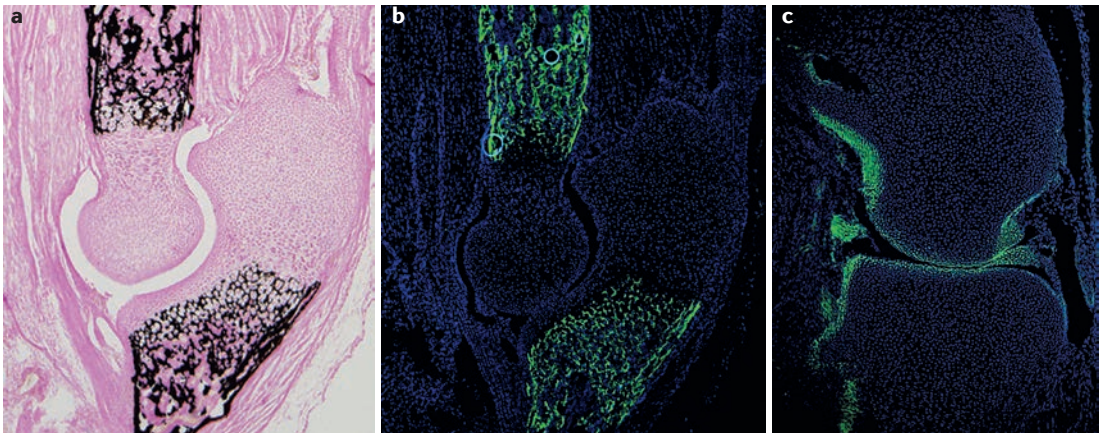

Figure 7 | **Bone morphogenetic protein signalling domains in knees versus elbows.** Longitudinal sections from forelimbs **a** | and **b** | or hindlimbs **c** | show the elbow and knee joints in newborn *BRE:Gfp* mice. Sections stained with Von Kossa and Fast Red counterstain (**a**) or 4′,6-diamidino-2-phenylindole counterstain (**b** and **c**).

In this Review, we have highlighted the involvement of BMP signalling in bone and joint formation, two key aspects of skeletal development. We discussed deranged BMP signalling as the aetiology of multiple skeletal diseases and reviewed the utility of BMPs as therapeutic agents in bone repair, providing current thinking on the importance of BMP signalling in osteoarthritis and regeneration of joint tissues.

Much remains to be learned. One concept that deserves further attention is the idea that a balance between TGF-β-like signals and BMP-like signals contributes to tissue homeostasis. This type of balance might be affected by the ratio of ligands such as BMP versus activin and/or myostatin to modulate muscle mass[224–226], or the profile of type II receptors shared by BMPs and activins to modulate bone mass[42]. Studies investigating how thresholds of signalling activity affect cell fate and tissue morphogenesis in the skeleton are also needed as accumulating data indicates that related ligands exhibit highly variable agonist activity despite similar binding affinities for a single receptor. For example, GDF3, GDF5 and BMP2 have similar affinities for BMPR1B, but the agonist activity of GDF3 and GDF5 is substantially lower than that of BMP2 (REFS 143,173,193,227). Thus, conditions that change the binding kinetics or agonist activity between BMPs, GDFs and BMPR1B might have biological importance. Furthermore, mutations affecting these relationships might have pathobiological significance. Efforts to correlate delayed versus accelerated chondrogenesis with particular mutations underlying various types of osteochodrodysplasias will help clarify this important, but unresolved, issue. This information is also likely to provide insight into how BMPs and GDFs coordinate to control joint morphogenesis, and could help establish a new framework for strategies to repair cartilage in patients with joint trauma or disease.

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Reference 227 shows that GDFs and BMPs have distinct agonist activity profiles depending on the profile of type I and type II receptors expressed by the target cell type.

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The authors declare no competing interests.

Review criteria
Original full-text research or review articles published between 1950 and 2015 and available in English through the Countway Library at Harvard Medical School were identified on PubMed and OMIM databases by using each ligand, type I receptor, type II receptor, SMAD and a subset of secreted antagonists as individual search query keywords. We also searched the reference lists of identified articles for further relevant papers.

SUPPLEMENTARY INFORMATION
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Supplementary Table 1 | Experimental genetics of BMP pathway ligands in murine skeletal development

Genotype (Ligands)		Phenotype
Bmp2$^{-/-}$		Embryonic lethal prior to skeletogenesis. Delayed primitive streak, small allantois, lack of amnion, heart defects[1]. Decreased primordial germ cells[2]
Bmp2$^{flox/flox}$	*Prx1–Cre*[3]	Periosteal growth defect. Brittle bones. Spontaneous fractures. Inability to initiate fracture repair. Unable to host or donate bone grafts[4–6]
	Gdf5–Cre[7]	In progress (S. P. Pregizer, unpublished work)
	Col2A1–Cre[8]	Low bone mass. Delayed fracture healing[9]
	Col2A1–CreERT2[10]	Tamoxifen at E12.5: Defective chondrocyte proliferation and maturation[11]
	3.6kb-Col1A1–Cre[12]	Low bone mass and BMD. Periosteal growth defect. Narrow, brittle bones, reduced load to fracture. Poor vascularization of periosteum and marrow. Severe reduction in BMSC[13]. Defects in ondontogenesis and enamel formation[14]
	Osx1–Cre::GFP[15]	Brittle bones and teeth. Hypomineralization. Periosteal growth defect. Reduced load to fracture[16–18]
	2.3kb-Col1A1–Cre[19]	Normal bone mass and fracture healing[9]
Bmp4$^{-/-}$		Null: Embryonic lethal. No mesoderm. Het: cystic kidney, cranial malformations, microphtalmia. Polydactyly; low PGCs[20,21]
Bmp4$^{flox/flox}$	*Prx1–Cre*	Bone mass and fracture repair normal. Potential delay of patellar development[107], but fully formed in adults[5,22,23]
	Col2A1–CreERT2	Minor defects in chondrocyte maturation[11]
Bmp2$^{+/-}$; *Bmp4*$^{+/-}$		Fewer PGCs than *Bmp4*$^{+/-}$ or *Bmp2*$^{+/-}$ mutants[2]
Bmp2$^{flox/flox}$; *Bmp4*$^{flox/flox}$	*Prx1–Cre*	Hypertrophic cartilage forms but is not vascularized, resorbed, or converted to bone (arrested at bone collar). Missing digits. Fusion of zeugopod/autopod. Soft tissue syndactyly[5]
	Col2A1–CreERT2	Tamoxifen E12.5: Defective chondrocyte proliferation and maturation[11]
Bmp2$^{flox/flox}$; *Bmp4*$^{flox/flox}$; *Bmp7*$^{flox/flox}$	*Msx2–Cre*[24]	Severe defects in digit specification[25]
Bmp3$^{-/-}$		High bone mass[26]
Bmp3b$^{-/-}$ *(Gdf10)*		Normal[27]
Bmp5$^{-/-}$		Reduced length and width of long bones. Short ears. Lung and kidney defects[28–31]
Bmp6$^{-/-}$		Delayed sternum ossification[32]
Bmp6$^{-/-}$; *Bmp5*$^{-/-}$		Enhanced sternal defects versus *Bmp6*$^{-/-}$[32]
Bmp7$^{-/-}$		Perinatal lethality. Skeletal defects in skull, ribs, hindlimb, and unilateral polydactyly[33]. Eye defects and kidney agenesis[34]
Bmp7$^{flox/flox}$	*Prx1–Cre*	Normal skeletal development[5, 35]. Osteoarthritis[36]
Bmp7$^{flox/flox}$; *Bmp2*$^{flox/flox}$	*Prx1–Cre*	Similar to *Bmp2; Prx1–Cre*, but smaller. Deformed scapula and fibula[5]
Bmp7$^{+/-}$; *Bmp4*$^{+/-}$		Skeletal defects in rib cage and limb[37]
Bmp7$^{-/-}$; *Bmp5*$^{-/-}$		Embryonic lethal. Defects in allantois, heart, branchial arches, and

	forebrain[38]
Bmp7$^{-/-}$; *Bmp6*$^{-/-}$	Embryonic lethal at E10.5. Cardiac cushion, septation defects[39]
Bmp7$^{-/-}$; *Alk6*$^{-/-}$	Severe hypoplasia or aplasia of most structures in autopod and zeugopod[40,41]
Bmp8a$^{-/-}$	Defects in spermatogenesis and epididymis[42]
Bmp8a$^{-/-}$; *Bmp7*$^{+/-}$	More severe defects in spermatogenesis than *Bmp8a*$^{-/-}$[43]
Bmp8a$^{+/-}$; *Bmp8b*$^{+/-}$	Defects in spermatogenesis and epididymis[42]
Bmp8b$^{-/-}$	Short allantois, defects in PGCs, testis, and spermatogenesis[44,45]; posterior truncation, heart defects and lack of optic vesicle[45]
Bmp9$^{-/-}$ *(Gdf2)*	Impaired vascular development[46]
Bmp10$^{-/-}$	Embryonic lethal. Vascular development defects. Heart defects[47]
Gdf1$^{-/-}$	Defects in left/right symmetry[48]
Gdf3$^{-/-}$	Resistant to high-fat diet induced obesity[49]
Gdf5$^{-/-}$ *(Cdmp1/Bmp14)*	Defects in limb bud outgrowth[50] and growth plate chondrogenesis[51]. Brachypodism (short limb bones and absence of some distal joints in wrists/digits)[40,52,53]
Gdf5$^{-/-}$; *Bmp5*$^{-/-}$	Brachypodism plus joint defects in sternebrae and ribs[53]
Gdf5$^{-/-}$; *Alk6*$^{-/-}$	Enhanced brachypodism[40]
Gdf5$^{-/-}$; *Gdf6*$^{-/-}$	Severe reduction or loss of limb bones, more joints missing than in single mutants; scoliosis, intervertebral cartilage defects[54]
Gdf6$^{-/-}$ *(Cdmp2, Bmp13)*	Joint, ligament, cartilage defects in wrists/ankles, and middle ear[54]
Gdf7$^{-/-}$ *(Bmp12)*	Accelerated growth plate chondrogenesis[55]. Defects in spinal cord[56] and seminal vesicle[57]
Gdf8$^{-/-}$ *(Myostatin)*	Skeletal muscle hypertrophy[58]; osteoarthritis with more osteoclasts, inflammatory joint destruction[59]; brittle tendons[60,61]
Gdf9$^{-/-}$	Subfertile due to defects in oogenesis[62]
Gdf9$^{+/-}$; *Bmp15*$^{-/-}$	Exacerbated defects in oogenesis versus *Bmp15*$^{-/-}$[62]
Gdf11$^{-/-}$ *(Bmp11)*	Anterior homeotic transformations in the axial skeleton. Posterior displacement of hindlimbs[63]. Inhibits chondrogenesis in chick[64]
Tgfβ1$^{-/-}$	Postnatal lethal hyper immunity[65]; angiogenesis defects[66]
Tgfβ1$^{-/-}$; *Rag2*$^{-/-}$	Do not maintain adult bone mass, osteoblast migration defect[67]
Tgfβ2$^{-/-}$	Perinatal lethal. Cleft palate. Limb, craniofacial and rib and sternum defects. Short radius/ulna[68,69]
Tgfβ3$^{-/-}$	Perinatal lethal. Cleft palate[70]
Tgfβ2$^{-/-}$; *Tgfβ3*$^{-/-}$	Loss of tendons and ligaments[71]
ActivinβA$^{-/-}$ *(InhbA)*	Lack whiskers, lower incisors/molars; cleft palate[72]
ActivinβB$^{-/-}$ *(InhbB)*	Eyelid and female reproductive defects[73]
ActivinβC$^{-/-}$ *(InhbC)*	Viable; no obvious abnormalities[74]
ActivinβE$^{-/-}$ *(InhbE)*	Viable; no obvious abnormalities[74]
Inhibinα$^{-/-}$	Ovarian cancer; infertility; adrenal tumors; cachexia[75]
Nodal$^{-/-}$	Embryonic lethal. Arrested egg cylinder, no primitive streak or mesoderm[76,77]. Skeletal defects in cKO unknown
Nodal$^{+/-}$; *Smad2*$^{-/-}$	Gastrulation/left-right patterning defects; cyclopia[78]
Amh$^{-/-}$ *(Mis)*	Pseudo-hermaphrodites[79]

Descriptions of experimental genetics include null, conditional null, and knock-in alleles to the endogenous loci. Transgenic models based on over expression of endogenous genes by non-endogenous promoters were not included, as transgenic expression domains do not necessarily reflect natural expression domains, making phenotype interpretations difficult. BMSC, bone marrow stromal cell; E, embryonic; KO, knockout; PGC, primordial germ cell.

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Supplementary Table 2 | Experimental genetics of BMP pathway type I receptors in murine skeletal development

Genotype (Type I receptors)		Phenotype
Alk1$^{-/-}$ *(Acvrl1)*		Embryonic lethal, angiogenesis defects[1]
Alk2$^{-/-}$ *(Acvr1)*		Embryonic lethal. Defects in mesoderm formation as a result of defective visceral endoderm[2,3]; left right patterning defects[4]
Alk2$^{flox/flox}$	*Col2A1–Cre*	Cranial and vertebral hypoplasia, defects in segmentation and ossification; adult onset kyphosis[5]
Alk2$^{R206H/+}$		Digit malformations in hindlimb; inflammation and apoptosis of skeletal muscle; extraskeletal endochondral bone formation[6]
Alk3$^{-/-}$ *(Bmpr1a)*		Embryonic lethal. Epiblast proliferation defects, no mesoderm[7,8]
Alk3$^{flox/flox}$	*Brn–Cre*	Defective dorsal/ventral patterning in the limb[9]
	aMHC–Cre	Cardiac cushion defects[10]
	Prx1–Cre	Short limbs, loss of autopod, hypomineralization of parietal/interparietal bones, partially split sternum[11,12]
	Gdf5–Cre	Dispensable for development of most joints. Osteoarthritis, postnatal loss of articular cartilage[13].
	Agc1–CreERT2[14]	Arrest of postnatal longitudinal bone growth and absence of trabecular bone. Growth plate cartilage replaced by bone[15]
	Col2A1–Cre	Perinatal lethality with chondrodysplasia. Delayed ossification[16]. Thoracic vertebral defects[5]
	3.2kbCol1–CreER[17]	High bone mass due to low resorption/bone turnover[18,19]
	OG2–Cre[20] *(Ocn)*	High bone mass with low bone turnover[21]
Alk4$^{-/-}$ *(Acvr1b)*		Embryonic lethal. Arrest at egg cylinder, no mesoderm, similar to Nodal knockout[22]
Alk5$^{-/-}$ *(Tbr1)*		Embryonic lethal. Angiogenesis defects[23]
Alk5$^{flox/flox}$	*Dm1–Cre*[24]	Joint formation defects; short, wide bones; protrusion of ectopic cartilage into perichondrium; scoliosis/kyphosis[25]
Alk6$^{-/-}$ *(Bmpr1b)*		Comparable but less severe phenotype than *Gdf5*$^{-/-}$; brachypodism; chondrodysplasia; short bones; GDF5^{+} domains expanded at the expense of cartilage; Defects in seminal vesicle development, female reproduction[26,27]
Alk6$^{-/-}$*; Gdf5*$^{-/-}$		Close phenocopy of *Gdf5*$^{-/-}$ (most joints form except some distal sites in wrist/digits) plus additional sternal and tarsal fusions[26,27]
Alk6$^{-/-}$*; Bmp7*$^{-/-}$		Severe hypoplasia or aplasia of most structures in autopod and zeugopod[26,27]
Alk2$^{flox/flox}$*; Alk3*$^{flox/flox}$	*Prx1–Cre*	Loss of skeletal structures distal to ulna, rudimentary pelvic bones, fully split sternum[12]
Alk3$^{flox/flox}$ *; Alk6*$^{+/-}$	*Prx1–Cre*	
Alk2$^{flox/flox}$*; Alk3*$^{flox/flox}$*; Alk6*$^{+/-}$	*Prx1–Cre*	Loss of skeletal structures in the forelimb and hindlimb[12]
Alk2$^{flox/flox}$*; Alk3*$^{flox/flox}$	*Col2A1–Cre*	Perinatal lethal chondrodysplasia[5]
Alk2$^{flox/flox}$*; Alk6*$^{-/-}$	*Col2A1–Cre*	Perinatal lethal chondrodysplasia[5]
Alk3$^{flox/flox}$*; Alk6*$^{-/-}$	*Col2A1–Cre*	Endochondral ossification is blocked at condensation/ pre-chondrogenesis. Growth plate does not form[16]
Alk7$^{-/-}$ *(Acvr1c)*		Viable and fertile; no obvious skeletal defects; do not phenocopy *Nodal*, *Acvr2a*, or *Acvr2b* null mice[28]

Descriptions of experimental genetics include null, conditional null, and knock-in alleles to the endogenous loci. Transgenic models based on over expression of endogenous genes by non-endogenous promoters were not included, as transgenic expression domains do not necessarily reflect natural expression domains, making phenotype interpretations difficult.

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Supplementary Table 3 | Experimental genetics of BMP pathway type II receptors in murine skeletal development

Genotype (Type II receptors)		Phenotype
Bmpr2$^{-/-}$		Embryonic lethal. Arrest at egg cylinder. No mesoderm[1]
Bmpr2$^{flox/flox}$	*Prx1–Cre*	Dispensable for development of limb skeleton[2]. Adult-onset high bone mass due to high osteoblast activity[3]
Acvr2a$^{-/-}$		Reproductive defects with low FSH. Mild developmental skeletal defects reminiscent of Pierre-Robin syndrome[4]
Acvr2b$^{-/-}$		Defects in axial patterning; 45% right isomerism[5]; periosteal defect and enhanced chondrogenesis[6]
Acvr2a$^{-/-}$; *Nodal*$^{+/-}$		Rostral head defects, cyclopia, and right isomerism[7]
Acvr2a$^{-/-}$; *Acvr2b*$^{+/-}$		Anterior neural truncation and defective elongation of primitive streak[7]
Acvr2a$^{+/-}$; *Acvr2b*$^{-/-}$		Vertebral transformations, no tail, cyclopia, right isomerism[8]
Acvr2a$^{-/-}$; *Acvr2b*$^{-/-}$		Embryonic lethal. Arrest at egg cylinder. No mesoderm[7]
Tgfbr2$^{-/-}$		Embryonic lethal at ~E10.5 with angiogenesis and hematopoietic defects[9]
Tgfbr2$^{flox/flox}$	*Prx1–Cre*	Loss of some phalangeal joints, accelerated chondrogenesis, expanded cartilage at the expense of interzone. Short bones[10–12], patellar aplasia[13], loss of tendons and ligaments[14]
	Col2A1–Cre	Joint defects in costal and vertebral sites, loss of intervertebral disks; normal developmental chondrogenesis and long bone formation, but postnatal longitudinal growth defect[15]
Amhr2$^{-/-}$ *(MisrII)*		Pseudohermaphrodites[16]

Descriptions of experimental genetics include null, conditional null, and knock-in alleles to the endogenous loci. Transgenic models based on over expression of endogenous genes by non-endogenous promoters were not included, as transgenic expression domains do not necessarily reflect natural expression domains, making phenotype interpretations difficult. E, embryonic; FSH, follicle-stimulating hormone.

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Supplementary Table 4 | Experimental genetics of BMP pathway SMADs in murine skeletal development

Genotype (Smads)		Phenotype
Smad1$^{-/-}$		Defects in visceral endoderm, extraembryonic mesoderm, and reduced number or lack of primordial germ cells (PGCs)[1,2]
Smad1$^{flox/flox}$	*Col2A1–Cre*	Viable and fertile; joints form normally[3]; delayed calvarial ossification[4]
	Col1A1–Cre[5]	Osteopenia[4]
Smad2$^{-/-}$		Embryonic lethal at ~E8.5. Egg cylinder and mesoderm defects. Defects in visceral endoderm causing abnormal mesoderm formation and A-P patterning; mutant ES cells fail to contribute to gut endoderm; left-right patterning defects[6–10]
Smad3$^{-/-}$		Osteoarthritis; degeneration of articular cartilage; accelerated chondrocyte hypertrophy[11]. Metastatic colon cancer[12]. Impaired immune response; accelerated wound healing[13]
Smad4$^{-/-}$		Gastrulation defects, no mesoderm; gastric polyposis/colon cancer in heterozygotes[14–17]
Smad4$^{flox/flox}$	*Dm1–Cre*	Embryonic lethal (V. S. Salazar, unpublished work).
	Prx1–Cre	Loss of bones in parietal/interparietal zone, distal to the shoulder and pelvic girdles; split sternum; chondrocytes do not condense, become apoptotic, and fail to form digit ray primordia[18,19]
	Col2A1–Cre	Dwarfism; disorganized growth plate; accelerated chondrocyte differentiation; chondrocyte apoptosis; ectopic bone collar in perichondrium[20]
	Osx1–Cre[21]	Dwarfism; small, brittle bones and teeth with spontaneous fractures; defects in collagen biosynthesis; osteoblast apoptosis[22]
	Osx1–CreERT2[23]	Increased proliferation of osteoprogenitors[24]
	Ocn–Cre (OG2)	Dwarfism, low bone mass with hypomineralization associated with low bone turnover; delayed medullary vascularization[25]
Smad5$^{-/-}$		Embryonic lethal. Impaired angiogenesis in yolksac; reduced mesoderm; defects in primordial germ cell formation and left-right asymmetry; craniofacial defects[26–29]
Smad6$^{-/-}$		Cardiac valve hyperplasia and outflow tract separation defects[30]. Vertebrae and sternabrae defects; enhanced chondrocyte proliferation, hypertrophy, and apoptosis[31]
Smad7$^{-/-}$		Perinatal lethal. Scleroderma-like phenotype (autoimmune)[32]
Smad7$^{hypomorphic/hypomorphic}$		Axial and appendicular chondrocyte defects in cell cycle and terminal differentiation. Hypocellular growth plates due to increased apoptosis[33,34]
Smad8$^{-/-}$ *(aka Smad9)*		Viable and fertile[3]. Defective pulmonary remodeling[35]
Smad1$^{flox/flox}$; *Smad5*$^{flox/flox}$	*Col2A1–Cre*	Severe chondrodysplasia; poor vascularization; joints form normally[3]
Smad1$^{flox/flox}$ *Smad5*$^{flox/flox}$; *Smad8*$^{-/-}$	*Col2A1–Cre*	Severe chondrodysplasia, same as *Smad1/5; Col2A1–Cre*[3]

Descriptions of experimental genetics include null, conditional null, and knock-in alleles to the endogenous loci. Transgenic models based on over expression of endogenous genes by non-endogenous promoters were not included, as transgenic expression domains do not necessarily reflect natural expression domains, making phenotype interpretations difficult. E, embryonic; ES, embryonic stem; PGC, primordial germ cell.

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Supplementary Table 5 | Experimental genetics of BMP pathway secreted antagonists in murine skeletal development

Genotype (Antagonists)		Phenotype
Noggin$^{-/-}$		Null: Perinatal lethal; lack of all joints[1]; somite defects[2]. Heterozygote: Conductive hearing loss, kyphosis, rib deformities, ossification defects[3]; delayed progression of osteoarthritis[4]
Noggin$^{flox/flox}$	*Prx1–Cre*	Lack all appendicular and sternal joints (V. S. Salazar, unpublished work)
	Col2A1–Cre	Joint defects in wrists and digits[5]
	Gdf5–Cre	Joint defects in wrists and digits (L. W. Gamer, unpublished work)
	Osx1–Cre	Joint patterning is normal (V. S. Salazar, unpublished work)
	Ocn–Cre[6]	Joint patterning is normal. Low bone mass due to resorption[6]
Gremlin$^{-/-}$		Joint pattering is normal. Fusion of stylopod. Fewer digits[7]
Gremlin$^{flox/flox}$	*Ocn–Cre*	High bone mass[8]
Noggin$^{flox/flox}$; *Gremlin*$^{flox/flox}$	*beta-actin–Cre*	Fail to initiate sclerotome and axial skeleton[9]
Tsg$^{-/-}$		Slight delay in acquisition of peak adult bone mass[10]
Follistatin$^{-/-}$		Perinatal lethal; Craniofacial, skin defects; growth retardation[11]
Dan$^{-/-}$		No effect on limb outgrowth or patterning[12]
LeftyA/1$^{-/-}$		Abnormal left/right axis (left isomerism)[13]
LeftyB/2$^{-/-}$		Extended streak, excessive mesoderm, left isomerism[14]

Descriptions of experimental genetics include null, conditional null, and knock-in alleles to the endogenous loci. Transgenic models based on over expression of endogenous genes by non-endogenous promoters were not included, as transgenic expression domains do not necessarily reflect natural expression domains, making phenotype interpretations difficult.

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