The genetic basis for skeletal diseases

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We walk, run, work and play, paying little attention to our bones, their joints and their muscle connections, because the system works. Evolution has refined robust genetic mechanisms for skeletal development and growth that are able to direct the formation of a complex, yet wonderfully adaptable organ system. How is it done? Recent studies of rare genetic diseases have identified many of the critical transcription factors and signalling pathways specifying the normal development of bones, confirming the wisdom of William Harvey when he said: "nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path".

Genetic studies of diseases that affect skeletal development and growth are providing invaluable insights into the roles not only of individual genes, but also of entire developmental pathways. Different mutations in the same gene may result in a range of abnormalities, and disease 'families' are frequently caused by mutations in components of the same pathway. Correlating these clinical phenotypes with the identified molecular alterations provides a sensitive method for analysing structure–function relationships.

In many cases the alterations are in molecules that — based on biochemical analyses — could not have been reasonably suspected to be important in bone biology. Such surprising discoveries are opening doors to new research areas. Because the gene mutations are often not simple loss-of-function mutations, they provide the impetus for introducing specific mutations into mice to generate models for studies of pathogenesis and fundamental aspects of cell differentiation and function.

Given the space limitations of this review, we have focused on examples that illustrate how studies of disease families are contributing to the understanding of regulatory pathways. We have excluded diseases (such as disorders of extracellular matrix components and various craniosynostosis syndromes) that have been discussed in recent reviews[1–3]. Human studies provide important information that complements data from experimental analyses of gene function in animals and cells. Although experimental studies are clearly of enormous importance, examining the phenotypic consequences of gene mutations in inbred mouse strains provides a view of gene function under conditions of minimal genetic and environmental variability. In contrast, studies of gene mutations in human families and populations can provide insights into adaptability of genetic mechanisms and biological systems within a complex environment. A full understanding of the genetic mechanisms for bone assembly, growth and function provided by evolution will be possible only by utilizing information from all these types of studies. This requires open communication between clinical geneticists, biochemists, and cell and developmental biologists, and we hope this brief review will stimulate that communication.

Classification of skeletal diseases

The vertebrate skeleton is formed by mesenchymal cells condensing into tissue elements outlining the pattern of future bones (the patterning phase). This is followed by differentiation to cartilage cells (chondrocytes) or bone cells (osteoblasts) within the condensations. Subsequent growth during the organogenesis phase generates cartilage models (anlagen) of future bones (as in limb bones) or membranous bones (as in the cranial vault) (Fig. 1). The cartilage anlagen are replaced by bone and marrow in a process called endochondral ossification. Finally, a process of growth and remodelling after birth (in a growth and maintenance phase) results in a skeleton that is well adapted to its function as an organ not only for movement, but also for support and protection of internal organs, blood cell production and regulation of calcium homeostasis.

Mutations in early patterning genes cause disorders called dysostoses; these affect only specific skeletal elements, leaving the rest of the skeleton largely unaffected. In contrast, mutations in genes that are involved primarily in organogenesis cause disorders called osteochondrodysplasias, which affect the development and growth of most skeletal elements in a generalized fashion[4]. Many genes have important functions in both these processes such that some inherited disorders can display features of both dysostosis and osteochondrodysplasia[5]. Genes used during skeletal development may also be important in other organogenetic events so that when mutated, the resulting skeletal genetic defects are part of a syndrome that may also include defects in nonskeletal tissues.

Disorders of bone and cartilage cell differentiation

Disorders affecting differentiation of cartilage and bone cells have features of both dysostosis and osteochondrodysplasia. Studies of two such disorders, campomelic dysplasia (CD; Mendelian Inheritance in Man (MIM) database no. 114290) and cleidocranial dysplasia (CCD; MIM 119600), have led to insights into the transcriptional machinery responsible for differentiation of chondrocytes and osteoblasts (Fig. 2).

The study of the dominant disorder CD represents an excellent example of how identification of human disease genes can lead to fundamental insights into the cellular basis for skeletal development. The discovery that loss-of-function mutations in the transcription factor SOX9 cause CD was followed by studies that comprise most of our current knowledge of how chondrocytic differentiation is transcriptionally regulated[6,7]. Babies with CD usually die during the perinatal period or in early infancy with skeletal abnormalities that strongly suggest a defect in cartilage, as well as in patterning of specific bones. Airway cartilage is defective, the rib case is small with a decreased number of ribs, the vertebral column is abnormal, and the base of the skull (formed

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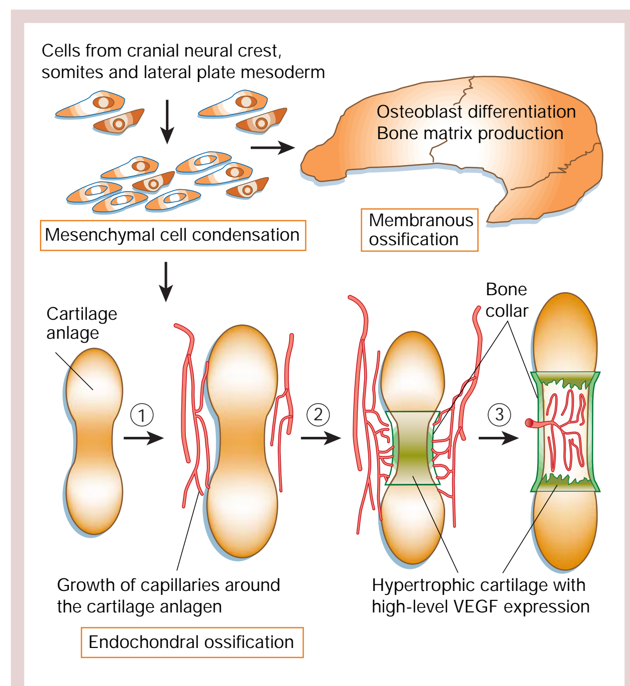

Figure 1 Skeletal development. Cells from cranial neural crest, somites and lateral plate mesoderm form mesenchymal condensations at sites of future bones. In membranous ossification, differentiation of mesenchymal cells to osteoblasts and production of bone matrix occurs directly, whereas in endochondral ossification, differentiation to chondrocytes and formation of cartilage models (anlagen) occurs first, followed by replacement of the models by bone. In step one, capillaries grow around cartilage anlagen. In step two, cells around the anlage differentiate to osteoblasts and produce a collar of bone around the middle of the cartilage. Chondrocytes in the centre of the cartilage mature to hypertrophy and express high levels of vascular endothelial growth factor (VEGF), a factor needed for invasion of capillaries into the cartilage. In step three, hypertrophic cartilage is replaced by marrow and bone and growth plates are formed (see review in this issue by Kronenberg, page 332).

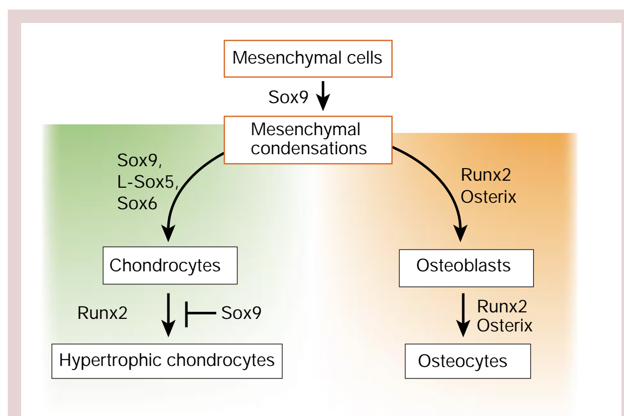

Figure 2 Differentiation factors in chondrocytic and osteoblastic differentiation. Sox9, together with Sox5 and Sox6, regulates differentiation of chondrocytes. Runx2 and Osterix control osteoblastic differentiation. Maturation of chondrocytes to hypertrophy is controlled positively by Runx2 and negatively by Sox9. As Runx2 has a role in both chondrocytic and osteoblastic differentiation, Osterix is likely the factor that provides specificity in osteoblastic differentiation.

through endochondral ossification) is small. Many of the generalized defects are similar to defects caused by mutations in the cartilage collagens II and XI (ref. 1).

Heterozygous *Sox9*-null mice (*Sox9*$^{+/-}$) display most of the abnormalities seen in CD patients[8]. Mutant mice die at birth with cleft palate and abnormal bending of endochondral bones in the limbs. Changes in the growth plates of *Sox9*$^{+/-}$ embryos suggest that Sox9 may inhibit the maturation of proliferating cells to hypertrophic chondrocytes. Inactivation of Sox9 in limb buds before mesenchymal condensations are formed results in complete absence of cartilage and bone[9]. In contrast, inactivation after condensations are formed results in defects similar to those reported for mice with inactivated *Sox5* and *Sox6* alleles[10]. Coupled with studies showing that Sox9 can bind to and activate the promoters/enhancers of chondrocyte-specific genes[11], these studies show that Sox9 is an important regulator of differentiation and maturation of chondrocytes (Fig. 2).

Cleidocranial dysplasia is a dominant condition characterized by defects in both endochondral and intramembranous bone formation, such as small or absent clavicles, fontanelles that do not fill in with bone, supernumerary teeth and short stature. The discovery of the transcription factor CBFA1/RUNX2 (core binding factor a1/runt homeodomain protein 2) as a critical regulator of bone formation and the cause of CCD in both humans and mice represents one of those key events when independent efforts in several laboratories lead to a confluence of discoveries highlighting one specific molecular process. In this case, several parallel avenues of research, namely, on transcriptional regulation of the bone protein osteocalcin[12], on mice with inactivated *Runx2* alleles aimed at determining a possible role for this factor in T-cell development[13,14], and on mutational analyses of mice and human patients with CCD[15], all contributed to the conclusion that RUNX2 is a critical transcription factor for osteoblastic differentiation and chondrocytic maturation (Fig. 2). Patients with CCD are heterozygous for loss-of-function mutations in RUNX2.

In the complete absence of Runx2 activity, as in Runx2-deficient mice, the entire skeleton consists only of cartilage, most of which is composed of resting or proliferating chondrocytes, or connective tissue membranes with no ossification[16]. In some limb cartilages of the endochondral skeleton, hypertrophy of chondrocytes is absent. But Runx2 cannot be absolutely necessary for chondrocytic maturation, as cartilage anlagen do show hypertrophy in the distal limbs (tibia, fibula, radius and ulna)[16]. The reason for this difference in sensitivity of chondrocytic hypertrophy to loss of Runx2 function in different bones is unknown. As two other members of the Runx family, Runx1 and Runx3, are expressed in growth-plate chondrocytes, it is possible that they can compensate for the loss of Runx2 in distal limbs. All three Runx factors form heterodimers with a common subunit, core-binding factor β (Cbfβ), recently demonstrated to be crucial for bone formation[17–19]. In the absence of Runx2, heterodimers of Cbfβ with Runx1 and/or Runx3 may be sufficient to allow cartilage hypertrophy in distal limbs.

The detailed pathways downstream of Runx2 that control osteoblastic differentiation are not known, but one critical mediator of Runx2 action is Osterix (Osx), a zinc finger-transcription factor expressed in osteoblasts[20]. Osx-deficient mice exhibit a complete block in osteoblastic differentiation but no overt abnormality in chondrocyte differentiation and maturation (Fig. 2).

Dysostotic defects of patterning and cell differentiation

Dysostoses can be divided into three groups, reflecting the different origins of the progenitor cells and differences in the developmental processes by which skeletal elements are formed in the craniofacial, axial and limb skeleton (Fig. 3).

Craniofacial patterning and differentiation defects

Craniofacial dysostoses include abnormalities in migration and/or differentiation of cranial neural crest cells (see review in this issue by Helms and Schneider, page 326). Analyses of variants of one of these disorders, Waardenburg syndrome, together with relevant mouse

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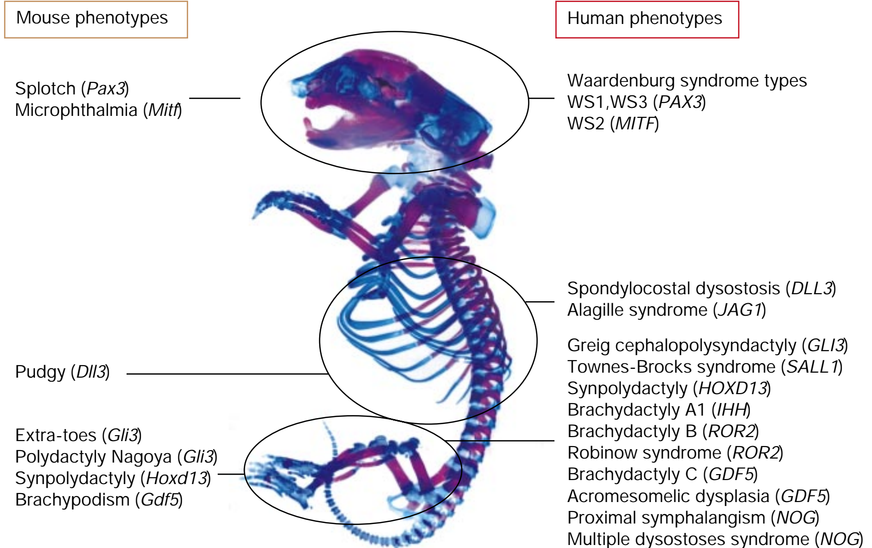

Figure 3 Mouse and human phenotypes caused by mutations affecting skeletal patterning and differentiation. The grouping of the disorders reflects the different origins of the progenitor cells in the craniofacial (cranial neural crest), axial (somites) and limb skeleton (lateral plate mesoderm). Only disorders discussed in the text are listed. The responsible genes are in parentheses after the names of the syndromes.

models, have greatly advanced the understanding of regulation of neural crest cell differentiation, migration and interactions and illustrate how disease families can help elucidate important developmental pathways. The skeletal manifestations of Waardenburg syndrome include patterning defects in craniofacial and limb bones. In addition, one of the responsible genes encodes a component of a transcription factor network involved in differentiation of multinucleated bone-resorbing cells, osteoclasts, from mononuclear progenitors. For this reason we briefly describe three of the four clinical types (WS1–WS4) of Waardenburg syndrome, which is characterized by deafness and pigmentation abnormalities in variable combination with skeletal anomalies and congenital megacolon.

WS1 (MIM 193500) and WS3 (MIM 148820) are caused by mutations in *PAX3*, a member of a family of transcription factors containing two interdependent DNA-binding domains — a homeodomain and a paired domain[21]. *PAX3* is expressed in somites and the dorsal region of the neural tube where neural crest cells start their migration[22]. In humans, several different *PAX3* mutations can cause abnormalities in craniofacial bone and soft tissues, spina bifida and cleft lip/palate. PAX3 is also an important regulator of the migration of precursor cells for muscle into the developing limbs[23]. Patients with WS3 show limb defects such as fusion of wrist bones, syndactyly, and small or missing phalangeal bones. In the mouse, point mutations or deletions in *Pax3* cause the Splotch (Sp) phenotype[24].

One of the targets of PAX3 is Microphthalmia (MITF), a member of the MITF-TFE family of basic helix-loop-helix- and leucine zipper-containing transcription factors[25]. Mutant PAX3 proteins associated with WS1 fail to activate the MITF promoter. Heterozygous mutations in *MITF* are found in many patients with WS2 (MIM 193510)[26]. Mice with mutations in *Mitf* (*mi*) serve as models for WS2 (ref. 27). In addition, and unlike the situation in humans, some of the strong dominant mutations in *Mitf* cause increased deposition of bone (osteopetrosis) in homozygous animals. This skeletal abnormality is due to a defect in the formation of bone-resorbing osteoclasts, caused by a block in colony stimulating factor-1 (CSF-1)-mediated signalling. Stimulation of mononuclear osteoclast progenitor cells by CSF-1 (in combination with RANK ligand, RANKL) leads to phosphorylation of Mitf and its homologue Tfe3. The two modified factors in turn associate with a transcriptional co-activator (p300/CBP) to stimulate formation of multinucleated osteoclasts[28] (Fig. 4). Osteoclastogenesis is normal in mice lacking either Mitf or Tfe3, but the combined loss of both transcription factors, results in severe lack of osteoclasts[29]. In addition to reduced osteoclast numbers in mice with dominant mutations in *Mitf*, expression of cathepsin K, essential for the ability of osteoclasts to degrade the organic matrix in bone, is also reduced. This is a consequence of the inability of mutant Mitf to activate the cathepsin K promoter[30].

Axial patterning and differentiation defects

Mutations associated with dysostoses of the axial skeleton affect somite development and cause abnormalities in the segmentation of the vertebral column (spondylocostal dysostosis; MIM 277300) and formation of caudal structures (sacral agenesis). Numerous studies show the importance of Notch signalling for segmentation of mesoderm into somites. A 'segmentation clock', involving the transcription factor Hairy 1, drives the expression of the glycosyl transferase Lunatic fringe (Lfng) and other components of the Notch–Delta pathway[31,32]. Mutations in the Notch ligand Delta-like3 (*Dll3*) cause disruption of the segmentation clock both in humans and mice. In families with a recessive form of spondylocostal dysostosis, associated with loss-of-function mutations in *DLL3*, affected individuals have short stature and vertebral and rib abnormalities[33]. Severe vertebral and rib deformities are also seen in an X-ray-induced mouse mutant, pudgy, caused by disruption of Dll3 function[34], and mice that are homozygous for targeted *Dll3*-null alleles have vertebral defects caused by perturbation of somite formation[35]. Mice with inactivated *Lfng* alleles display a phenotype similar to pudgy[36,37].

Mutations resulting in loss of function of the Notch ligand Jagged 1 (JAG1) are associated with the dominant Alagille syndrome (MIM 118450) in humans[38,39]. Affected individuals have abnormalities of the liver, heart, eye, facial structures, and other parts of the skeleton. Short distal phalangeal bones may be present, but the most striking anomaly is abnormally shaped vertebral bodies (butterfly vertebrae). In mice, Jagged does not seem to be required for somite formation. *Jag1*$^{+/-}$ mice have ocular defects similar to eye anomalies sometimes seen in Alagille syndrome patients. Mice that are doubly heterozygous for a *Jag1*-null allele and a hypomorphic *Notch2* allele exhibit all the nonskeletal components of Alagille syndrome, but the bone defects are not present[40,41].

Limb patterning and cell differentiation defects

Dysostoses of the limb skeleton include abnormalities of patterning of hands and feet and disorders of growth and joint formation. Many of the syndromes (polydactylies and syndactylies) are caused by

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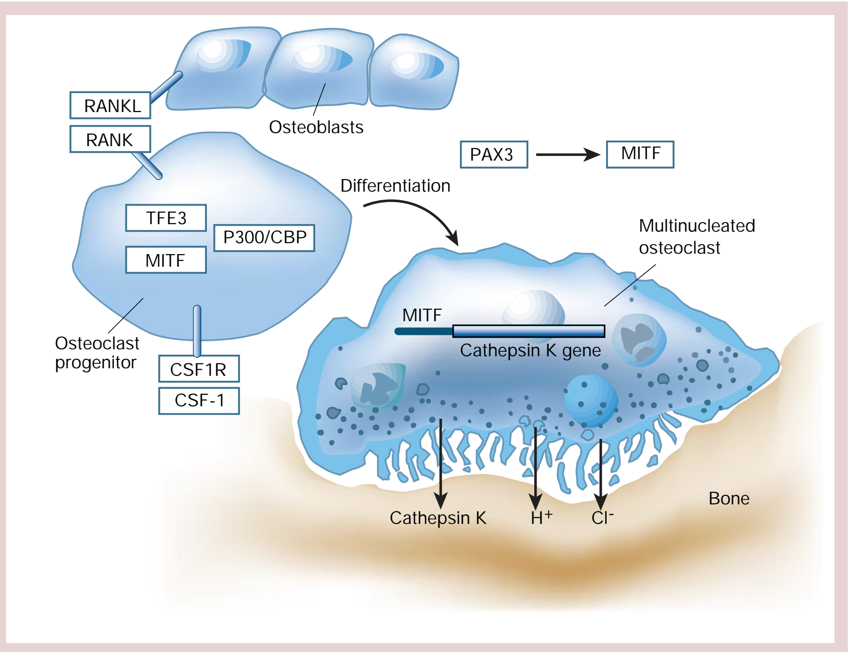

Figure 4 Genes causing different types of Waardenburg syndrome and their role in osteoclast differentiation. As described in the text, mutations in the genes encoding PAX3 and MITF cause WS1–WS3. MITF is a critical transcription factor for differentiation of osteoclast progenitors to multinucleated osteoclasts and for activation of the gene encoding cathepsin K, a secreted enzyme required for efficient degradation of bone matrix. As MITF is a downstream target of PAX3 in neural crest-derived cells, it is possible that PAX3 may also have a role in osteoclastogenesis.

mutations in components of the Sonic hedgehog pathway, reflecting the critical importance of Sonic hedgehog for patterning of the limb skeleton. Secreted by cells in the posterior zone of polarizing activity in the growing limb bud, Sonic hedgehog controls the expression of transcription factors such as Gli3 and Sall1, and the nested expression of members of the Hoxd cluster (see review in this issue by Mariani and Martin, page 319). In Greig cephalopolysyndactyly (MIM 175700), deletions or truncations in the transcriptional repressor GLI3 cause broad thumbs, polydactyly and syndactyly, and craniofacial anomalies[42]. In Townes-Brocks syndrome (MIM 107480), mutations in the gene encoding SALL1, a zinc-finger transcription factor, result in anomalies (craniofacial, hand, renal and anal) in regions where hedgehog signalling is important during development[43]. A striking illustration of the importance of *Hoxd* gene expression is provided by synpolydactyly (MIM 186000). In individuals with in-frame expansions of a polyalanine tract in the amino-terminal region of HOXD13, there is syndactyly between fingers three and four and duplication of a finger in the syndactylous web in the hand[44]. Good mouse models for these patterning disorders include extra-toes (xt)[45] and polydactyly Nagoya (Pdn)[46], both caused by *Gli3* mutations, and synpolydactyly (spdh)[47], caused by mutations in *Hoxd13*.

Patterning genes control the formation and growth of individual skeletal elements in the limbs. For example, analyses of disorders that cause brachydactylies (shortening of fingers and toes) have identified Indian hedgehog (Ihh), receptor tyrosine kinase ROR2, and members of the bone morphogenetic protein (BMP) family as important downstream targets. Brachydactylies are divided into several types depending on which long bones and/or digits are affected.

Type A1 brachydactyly (MIM 112500), characterized by short middle phalanges of all digits, short thumbs and short stature, is of particular interest in that it was the first disorder to be described (by S. W. Farabee in his Harvard University Ph.D. thesis in 1903) as an autosomal dominant Mendelian trait. In several families, mutations have been identified in *Ihh*, at residues thought to be important for binding of Ihh to its receptor Patched[48]. Many of the features of the phenotype can be explained based on the role of Ihh in stimulating cell proliferation in growth-plate cartilage.

Brachydactyly type B (MIM 113000) patients, who have normal thumbs, but short fingers II–V, have dominant loss-of-function mutations in *ROR2* (ref. 49). That ROR2 has an important role in endochondral bone formation is evident from studies of Ror2 expression during mouse development and the phenotype of mice that are homozygous for *Ror2*-null alleles[50]. Ror2 is expressed in chondrocytes and perichondrial cells, and homozygous *Ror2*-null mice die around birth, with shortened snouts, limbs and tails, and cleft palate. Intriguingly, the severity of the cartilage defects increases in the distal parts of the limbs. Middle phalangeal bones are missing; a feature that is similar to what is seen in brachydactyly type B. Homozygosity for loss-of-function mutations in *ROR2* has been demonstrated in several families with recessive Robinow syndrome (MIM 180700). Affected individuals exhibit severe short-limbed dwarfism, rib, vertebral and other abnormalities and brachydactyly[51,52].

Dominantly inherited brachydactyly type C (MIM 113100), characterized by shortening of fingers II, III and V, short metacarpals and occasional fusion of the proximal finger joints, is caused by loss-of-function mutations in *GDF5/CDMP-1* (growth and differentiation factor 5/cartilage-derived morphogenetic protein-1), a member of the BMP/transforming growth factor-β family of cytokines[53]. Homozygosity for a null mutation in *Gdf5* results in mice with short legs (brachypodism)[54]. In humans, homozygosity for loss-of-function mutations in *GDF5* causes Hunter-Thompson type acromesomelic dysplasia (MIM 201250), a disorder with severe shortening of arms and legs and small digits, but with normal craniofacial and axial skeletons[55]. As is the case with *ROR2* mutations, there is a proximal–distal gradient of increasing severity in the limbs.

Proximal symphalangism (MIM 185500), occasionally seen as part of brachydactyly type C, can also be inherited as a distinct dominant disorder, often associated with early onset deafness. Identification of mutations in *NOG* — the secreted BMP-antagonist Noggin — explains the similarity to brachydactyly type C[56]. Noggin mutations have also been found in multiple dysostoses syndrome (MIM 186500), a disorder characterized by fusion of joints in hands and feet as well as elbows, hips and intervertebral joints[56]. Fusion of limb bones is among the many defects in mice homozygous for *Noggin*-null alleles[57].

Osteochondrodysplastic growth-plate defects

Osteochondrodysplasias represent a diverse group of malformations. Because they are caused by mutations in genes that affect the function of cartilage and bone tissues, it is not surprising that a large number of these disorders result in abnormal growth plate cartilage with consequent dwarfism.

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In growth plates, proliferation and maturation of chondrocytes and replacement of hypertrophic cartilage by bone marrow and bone is a highly regulated process. As discussed elsewhere in this issue by Kronenberg (page 332), Indian hedgehog (Ihh) and parathyroid hormone-like hormone (Pthlh, also called parathyroid hormone-related peptide or PTHrP) form a negative feedback loop that controls differentiation of chondrocytes to hypertrophy. In humans, a dominant disorder, Jansen's metaphyseal chondrodysplasia (MIM 156400), characterized by short limbs and increased bone turnover, is caused by mutations resulting in activation of the receptor (PTHR) for PTHLH[58]. Mutations that impair PTHR function result in Blomstrand's chondrodysplasia (MIM 215045), a recessive disorder characterized by early death and advanced bone maturation[59]. Mutations in *PTHR* have also been identified in individuals with benign cartilage tumours called enchondromas[60]. These can occur as solitary lesions or as multiple lesions in enchondromatosis (MIM 166000). The tumours are usually found in close proximity to growth plates. Consequently, it has been suspected that they result from abnormal regulation of proliferation and differentiation of chondrocytes. Hopyan *et al.*[60] have recently found a mutation in the extracellular domain of PTHR in two patients with enchondromatosis.

Ihh is a positive regulator of cell proliferation within growth plates (see review by Kronenberg, page 332). In contrast, fibroblast growth factor receptor 3 (Fgfr3) provides negative control. The first demonstration that Fgfr3 signalling is crucial in growth-plate physiology came from reports that mutations in *FGFR3* were responsible for several forms of dwarfism in humans[61–64]. All the mutations, including those causing the most common form of the disorder, achondroplasia (MIM 100800), result in various levels of activation of FGFR3 signalling[65]. Several mouse models have been made and these show phenotypes that correlate well with what is seen in the human syndromes. Nevertheless, much remains to be learned, as the genotype/phenotype correlation in disorders caused by *FGFR3* mutations is by no means clear. Not only do mutations located in different domains of the receptor cause different phenotypes, but different mutations in the same codon can also cause clinically distinct disorders. Thus, mutations in what is normally a lysine codon within the cytoplasmic tyrosine kinase activation loop of FGFR3 can result in three different syndromes — thanatophoric dysplasia (MIM 187600) when the lysine residue is replaced by glutamic acid or methionine, SADDAN syndrome (MIM 134934) when the lysine is replaced by methionine, and hypochondroplasia (MIM 146000) when the same lysine residue is replaced by asparagine or glutamine.

Within growth plates, expression of Fgfr3 is restricted to proliferating and hypertrophic zones. Because mice with inactivated *Fgf18* alleles have growth-plate alterations that are similar to those of mice with inactivated *Fgfr3* alleles[66–68], it has been suggested that Fgf18 is the physiological ligand for Fgfr3. How signalling through Fgfr3 by Fgf18 (or other FGFs) interacts with regulation of growth-plate activities by Ihh/Pthlh is not fully understood. However, recent experiments suggest that FGF signalling acts upstream of Ihh to regulate the onset of chondrocyte differentiation to hypertrophy[69].

Further insights into growth-plate regulation are provided by studies of Leri-Weill dyschondrosteosis (MIM 127300). Loss-of-function mutations in one allele of the transcription factor *SHOX* (for short stature homeobox gene), located at the tip of both X and Y (sex) chromosomes[70], results in a phenotype of short stature with shortening of the forearms and lower legs. Other loss-of-function mutations in *SHOX* have also been demonstrated in individuals with so-called idiopathic short stature and SHOX is probably responsible for the short stature in patients with Turner syndrome (absence of an X chromosome in females)[71]. Langer mesomelic dysplasia (MIM 249700), characterized by severe short stature with marked shortening of the limbs, is caused by loss-of-function mutations in *SHOX* on both sex chromosomes[72].

Histological study of abnormal growth plates that were surgically removed from the forearm of two patients with dyschondrosteosis showed shorter than normal columns of chondrocytes in the proliferating zone[73]. Within the columns, the chondrocytes were stacked side-by-side (not on top of each other as in normal growth plates) and formed nests of cells at various stages of maturation. SHOX activity may therefore control the stacking of chondrocytes within the proliferative zones of growth plates and maturation to hypertrophy. Also of interest is the outcome of an unusual 'experiment of nature'. In two families where two siblings with Langer mesomelic dysplasia each married a spouse with achondroplasia or hypochondroplasia, two children had achondroplasia or hypochondroplasia combined with Leri-Weill dyschondrosteosis, whereas two other children had Leri-Weill dyschondrosteosis[74]. A comparison of the affected individuals showed that the different phenotypes, when combined in the same individual, seemed to be milder than what one would expect if they would be additive. It is therefore possible that SHOX and FGFR3 are components of partially overlapping pathways within growth plates. For example, SHOX could act downstream of Pthlh/Ihh to control chondrocyte proliferation and maturation.

Future prospects

This brief review has described a small subgroup of a vast number of genetic diseases affecting the skeletal system. Other diseases could have been selected, but the main point to be made would not have been much different. These genetic disorders, apart from their compelling stories of human suffering and triumph, represent 'experiments of nature' with great potential for advancing the understanding of mechanisms underlying skeletal development.

With this in mind, where do we go from here? What are the significant questions in skeletal biology and what can studies of human genetic diseases contribute to the search for the answers? Several important questions come to mind, such as what are the mechanisms that specify the architecture of each and every bone in the skeleton, the processes of joint formation, and the proper insertion of ligaments and tendons? What are the cellular interactions that supply bones with blood vessels and nerves, and how do vessels and nerves support the growth and function of bones? A particularly important question is how cells build and remodel bones in postnatal life. Scientists have for centuries been impressed with the ability of bone cells to produce bone along trajectories of mechanical stress or in locations where maximal strength is obtained with a minimum of material; yet how this is accomplished is not known. Generations of students have marvelled at the ability of osteoblasts to deposit bone in spiralling lamellar layers around blood vessels, but how it is done remains obscure.

Answers to these questions will undoubtedly benefit from studies of gene disorders causing excessive lack or deposition of bone. The recent identification of loss- and gain-of-function mutations in the osteoblastic receptor LRP5 in families with low and high bone mass is likely to be followed by other exciting discoveries along the same line (see review in this issue by Harada and Rodan, page 349). Coupled with the mapping of quantitative trait loci in large cohorts of individuals, such studies will probably result in identification of components in interacting pathways that regulate skeletal remodelling, as well as in pinpointing sequence variations that contribute to biological variability. The insights to be gained may help improve methods for bone repair, facilitate tissue engineering of skeletal tissues, and allow development of new effective strategies to prevent and cure osteoporosis. □

doi:10.1038/nature01659

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