REVIEW ARTICLE
Genetic Disorders of the Skeleton: A Developmental Approach

Uwe Kornak and Stefan Mundlos
Institute for Medical Genetics, Charité University Hospital, Campus Virchow, Berlin

Although disorders of the skeleton are individually rare, they are of clinical relevance because of their overall frequency. Many attempts have been made in the past to identify disease groups in order to facilitate diagnosis and to draw conclusions about possible underlying pathomechanisms. Traditionally, skeletal disorders have been subdivided into dysostoses, defined as malformations of individual bones or groups of bones, and osteochondrodysplasias, defined as developmental disorders of chondro-osseous tissue. In light of the recent advances in molecular genetics, however, many phenotypically similar skeletal diseases comprising the classical categories turned out not to be based on defects in common genes or physiological pathways. In this article, we present a classification based on a combination of molecular pathology and embryology, taking into account the importance of development for the understanding of bone diseases.

Introduction

Genetic disorders affecting the skeleton comprise a large group of clinically distinct and genetically heterogeneous conditions. Clinical manifestations range from neonatal lethality to only mild growth retardation. Although they are individually rare, disorders of the skeleton are of clinical relevance because of their overall frequency. Their clinical diversity makes these disorders often difficult to diagnose, and many attempts have been made to delineate single entities or groups of diseases to facilitate diagnosis. Traditionally, skeletal disorders have been subdivided into dysostoses, defined as malformations of individual bones or groups of bones, and osteochondrodysplasias, defined as developmental disorders of chondro-osseous tissue. On the basis of clinical and radiological features, an international group of experts created a taxonomy called the “Nomenclature and Classification of the Osteochondrodysplasias.” In the most recent revision, the dysostoses have been incorporated into the nomenclature, which also has been called a “nosology” (Hall 2002). Although this nosology is based on clinical entities, it also takes into account the more recent advances in our understanding of the molecular pathology of skeletal conditions. In many instances, the molecular data support the original clinical delineation. In other cases, however, it results in the grouping of conditions that have a common molecular origin but that have little in common clinically. For example, mutations in \textit{COL2A1} can result in such diverse conditions as lethal achondrogenesis type II and Stickler dysplasia, which is characterized by moderate growth retardation, arthropathy, and eye disease. It is now becoming increasingly clear that several distinct classifications are needed that reflect, on one hand, the molecular pathology and, on the other, the clinical signs and symptoms. Several reviews of the rapidly changing molecular basis of osteochondrodysplasias have been published, focusing either on a molecular-pathogenetic classification (Mundlos and Olsen 1997; Superti-Furga et al. 2001) or on more specific aspects, such as transcriptional dysregulation (Hermanns and Lee 2001).

In this article, we present a classification, based on a combination of molecular pathology and embryology, that takes into account the importance of development for the understanding of bone diseases. This concept directly links the clinical phenotype to key cellular processes of skeletal biology, such as proliferation, differentiation, and apoptosis. Skeletal development starts with the formation of a pattern, a process during which the number, size, and shape of the individual skeletal elements are delineated. After the pattern is set, mesenchymal precursor cells migrate to the site of skeletogenesis, condense, and differentiate. Growth of the skeleton takes place primarily in the cartilaginous growth plates, highly specialized organs located at the ends of the long bones. Bone cells remain active after the growth phase has ended, to keep the skeleton in homeostasis by a process called “remodeling.” Accordingly, we have subdivided skeletal disorders into four major groups: disorders affecting skeletal patterning, condensation/differentiation...
of skeletal precursor structures ("anlagen"), growth, and homeostasis.

**Disorders of Skeletal Patterning**

The skeleton arises from three distinct sites. The axial skeleton, consisting of the vertebrae and ribs, originates from the somites; the appendicular skeleton has its origin in mesenchymal cells located in the lateral plate mesoderm. Most of the craniofacial bones are of neural-crest origin. Disorders affecting the craniofacial skeleton have been discussed in detail elsewhere (Wilkie and Morriss-Kay 2001) and will therefore not be presented here.

**The Axial Skeleton**

The axial skeleton, consisting of the vertebrae and the dorsal part of the ribs, is entirely derived from the somites, transient organizational structures of the developing embryo located on both sides of the neural tube. Somites are blocks of epithelial cells with a periodic structure that originate from the paraxial mesoderm. The formation of new somites and their detachment from the paraxial mesoderm have to occur in a highly ordered fashion simultaneously on both sides of the neural tube, in a craniocaudal direction (fig. 1A). Timed regulation of the formation and budding of new somites is given by oscillations of cycling genes that lead to waves of notch signaling sweeping up the paraxial mesoderm from the posterior to the anterior pole (Saga and Takeda 2001). In addition to this molecular clock, a stable gradient of Fgf8 expression from the posterior to the anterior pole of the embryo allows a spatial coordination of somite border formation. Dll proteins are notch ligands that reside at the cell surface. Their differential expression determines the size as well as the polarity of the somites. Any disturbance in this polarity results in abnormally spaced somites and in fusion of adjacent somites, as exemplified in the mouse mutant *pudgy*, which carries a mutation in *Dll3* (Kusumi et al. 1998).

As the somite moves rostrally, it matures and differentiates into the dermatomyotome—a structure giving rise to the entire appendicular and axial musculature, as well as to the dorsal epithelium—and the sclerotome, the primary origin of the axial skeleton (fig. 1B). The signaling molecule sonic hedgehog (Shh) is the major signal from the notochord/floor plate that initiates and controls sclerotome formation. It is evident that any disturbance of this process will result in abnormal anlagen of the vertebrae and thus in vertebral malformations.

**Conditions with Vertebral Malformations**

Abnormalities of the ribs and/or vertebrae are a relatively common finding in human malformation syndromes. Those that primarily affect the axial skeleton are summarized under the term “spondylocostal dysostoses” (SCDs). Although the causes of the majority of these genetically heterogeneous conditions remain undiscovered, one type of dominant SCD has been shown to be caused by mutations in *DLL3* (Bulman et al. 2000). Similar to the *pudgy* mutant in the mouse, which is caused by mutations in the mouse ortholog, *Dll3* (Kusumi et al. 1998), affected individuals show a wide variety of vertebral malformations, including fusions and half vertebrae.

Vertebral malformations are frequently observed in Robinow syndrome, a condition that will be discussed further below. Robinow syndrome is caused by mutations in ROR2, an orphan receptor tyrosine kinase (Afzal et al. 2000; van Bokhoven et al. 2000). The role of ROR2 in somite development remains to be determined.

**The Appendicular Skeleton**

The limb skeleton originates from the lateral plate mesoderm, which forms the limb bud as the result of a series of interactions with the overlying ectoderm. The mesenchymal cells of the growing limb bud begin to differentiate to form the various tissues of the limb in a proximodistal sequence, with structures being laid down progressively from a region of undifferentiated cells at the tip of the limb bud, known as the “progress zone.” The positional identity and thus differentiation of each cell is controlled by a three-dimensional coordinate system consisting of the dorsoventral, proximodistal, and anteroposterior axes. Each axis is controlled by a particular set of signaling molecules/pathways produced by a defined population of cells. Three signaling regions have been identified: the apical ectodermal ridge (AER), mediating limb bud outgrowth (proximodistal axis); ectoderm covering the sides of the bud, governing the dorsoventral pattern; and the zone of polarizing activity (ZPA), controlling the anteroposterior pattern (fig. 1C). Many of the signaling molecules that are produced by these signaling centers have been identified and characterized. Receptors have been identified, and intracellular signaling transduction pathways are being unraveled (Capdevila and Izpisua Belmonte 2001).

The AER is an anatomical structure consisting of densely packed ectodermal cells located at the very tip of the limb bud. Several different fibroblast growth factors (FGFs) are expressed and secreted by the AER and have been shown to be essential and sufficient to initiate and control outgrowth of the limb. FGF signaling is conveyed through the FGF receptors, which are expressed in the underlying mesenchyme. As discussed below, the FGF-signaling system is also important in later stages of development, when FGFs control skeletal morphogenesis and growth.

The ZPA is a region of mesenchyme located at the
Figure 1  Patterning of the axial and appendicular skeleton. A, Somitogenesis. Presomitic mesoderm on both sides of the neural tube becomes subdivided into somites in craniocaudal direction. To illustrate the spatial and temporal coordination of somite formation, a time difference of 90 min between the left and right portion of the presomitic mesoderm is depicted here. Waves of notch expression originating from the caudal pole of the embryo time the somite border formation so that a new somite is formed every 90 min. Spacial coordination is conveyed by a gradient of FGF8 expression. Gray shading of newly formed somites represents polarized expression ofDll3. Somites give rise to several structures, among them the axial skeleton. B, Dorsoventral patterning of the axial skeleton. Each somite subdivides into a dermamyotome, from which the muscles of the extremities are derived, and a sclerotome, which develops into the axial skeleton. This process is regulated by Shh, which is expressed by the notochord. C, Patterning of the limb buds. The AER is an important signaling center for proximodistal outgrowth of limb structures. p63 expression is crucial for sustaining the AER. The ZPA directs anteroposterior patterning by expression of Shh. Under the influence of Shh, the repressor form of Gli3, Gli3R, is converted to the activating form Gli3A, and a gradient of Shh and Gli3 expression is established. Expression of the Hoxd-cluster genes occurs in a highly ordered fashion as a result of the Shh/Gli3 gradient created by the ZPA. Wnt7a shows a polarized expression, which is strongest in the dorsal ectoderm of the limb bud. It induces Lmx1 in the underlying mesenchyme, which has a key role in dorsoventral patterning. D, The result of the patterning process becomes visible as mesenchymal condensations at later developmental stages. These condensations either develop into cartilaginous bone precursors in endochondral ossification or are directly transformed into bone in desmal ossification.
Polydactyly Disorders Involving the Hedgehog Pathway

Polydactyly is a relatively common malformation clinically subdivided into the preaxial (additional finger at the side of the thumb) and postaxial (additional finger at the side of the little finger) types. Recent evidence from mouse models has shown that many types of polydactyly directly or indirectly involve the hedgehog pathway. Mutations in \textit{SHH} itself, however, cause holoprosencephaly, a condition characterized by midline defects of the brain and the face with normal limbs. Mutations in the downstream effector of Shh, \textit{GLI3}, give rise to different phenotypes, the mildest of which are postaxial polydactyly type I (MIM 174200) and preaxial polydactyly type IV (MIM 174700) (Radhakrishna et al. 1997, 1999). Greig cephalopolysyndactyly (MIM 175700) is a dominant disorder that comprises hypertelorism and frontal bossing, as well as syndactyly and pre- or postaxial polydactyly of variable degrees (Vortkamp et al. 1991). The most-severe malformations are found in dominant Pallister-Hall syndrome (MIM 146510), a condition characterized by (i) malformations of the CNS and, occasionally, holoprosencephaly with midline clefting; (ii) craniofacial abnormalities; (iii) syndactyly and polydactyly (central and postaxial); and (iv) anal defects (Kang et al. 1997). No clear genotype/phenotype correlation exists.

Another protein that possibly acts downstream of Shh is the human homolog of the \textit{Drosophila spalt} gene, \textit{SALL1}. This zinc finger transcription factor has been found to be mutated in Townes-Brocks syndrome (TBS [MIM 107480]) (Kohlhase et al. 1998). Limb malformations include bifid or triphalangeal thumbs and preaxial polydactyly. Further characteristics are abnormal ears and urogenital, heart, and visceral abnormalities. In keeping with a role in anteroposterior patterning, \textit{Sall1} is expressed in the AER and the underlying mesenchyme. Regardless of the tremendous evolutionary distance from humans, the orthologous pathway in \textit{Drosophila} also coordinates anteroposterior patterning. The
exact targets of spalt and Sall1 are still elusive, but it was demonstrated that the Sall1 protein colocalizes with the telomere-binding protein PIN2 to pericentromeric heterochromatin (Netzer et al. 2001). Most mutations cluster in exon 2 of SALL1 and delete a zinc finger domain by truncating the protein, which is thought to render it nonfunctional.

Preaxial polydactyly is frequently associated with a triphalangeal thumb (a long fingerlike thumb with three phalanges instead of two). This condition, triphalangeal thumb–polysyndactyly syndrome (TPTPS [MIM 190605]), is inherited as an autosomal dominant trait with variable expressivity. It has been mapped to chromosome 7, close to the SHH gene (Heutink et al. 1994). Whereas no TPTPS mutations have been identified so far, an intronic deletion and insertion within the neighboring LMBR1 (Corf2) gene has recently been found in the human limb amputation condition acheiropodia and in the polydactylous mouse mutant sasquatch (Ssq), respectively (Ianakiev et al. 2001; Lettice et al. 2002). These findings and the subsequent identification of a translocation breakpoint, in a patient with postaxial polydactyly, in the same intron as the Ssq insertion (Lettice et al. 2002; Horikoshi et al. 2003) suggest the existence of a long-range cis enhancing element directing Shh expression in the limb. In the future, the identification of the underlying molecular defect of the mouse limb mutants Hemimelic extra toes (Hx) and Hammertoe (Hm), which have also been mapped to this syntenic region and display postaxial polydactyly plus tibial aplasia and syndactyly, may help to refine the genomic size of an Shh long-range limb enhancer element.

**Ectrodactyly and Other Absence/Hypoplasia Defects**

“Ectrodactyly” is a rather nonspecific descriptive term applied to a number of genetic and nongenetic malformations with absence deformities of the hands/feet. The great majority of these defects occur unilaterally and are nongenetic. The split hand/foot malformation (SHFM) is genetically heterogeneous and preferentially affects the central part of the distal limb (fig. 2). Similar phenotypes can be produced in the chick, by surgical removal of the AER. This results in truncation of the limbs at a position corresponding to the time point of AER ablation, indicating that SHFM may be caused by similar mechanisms.

The dominant ectrodactyly, ectodermal dysplasia, and clefting syndrome (EEC [MIM 604292]) was the first condition in which mutations in the p53 homolog p63 were identified (Celli et al. 1999). In addition to syndactyly and ectrodactyly of the hands and feet, cleft palate, hypodontia, and diverse signs of ectodermal dysplasia can be noted. EEC overlaps with limb-mammary syndrome (MIM 603543), in which the nipples are hypoplastic or absent, and nonsyndromic SHFM (MIM 605289), and all three disorders were shown to be allelic (Brunner et al. 2002). Expression of the p63 transcription factor is restricted to stem cells within the basal layer of squamous epithelia. Consequently, complete deletion of p63 in mice leads to an absence of squamous epithelia and their derivatives, such as hair, teeth, and mammary, lacrimal, and salivary glands. Moreover, limbs are severely truncated, which illustrates the utmost importance of functional ectoderm for limb bud initiation and patterning. Without p63, no AER is formed, and the mesenchymal cells of the limb bud degenerate, most likely because of a lack of FGF8 (Yang et al. 1999).

The human ectrodactyly disorders cannot simply be explained by a loss of p63 function. Even though all EEC mutations affect arginine residues in the DNA-binding domain, this does not necessarily entail a total loss of function, since p63 still can interact and thus influence other proteins via its SAM domain. Mutations in this domain, on the other hand, cause Hay-Wells syndrome (MIM 106260), which comprises only minor limb abnormalities. How exactly the changed DNA-binding capacity of p63 leads to a degeneration of only the central part of the AER to cause splitting of the autopod needs to be determined.

Another form of ectrodactyly has been linked to deletions on chromosome 7q (SHFM1 [MIM 183600]). This region contains two homeobox genes, DLX5 and DLX6. The deletion of both genes in the mouse results in an SHFM-like phenotype.

Other absence defects involve either the radial or the ulnar ray of the limb. Holt-Oram syndrome (HOS [MIM 142900]) is characterized by the combination of radial
ray defects, such as hypoplasia/aplasia of the thumb and aplasia of the entire radius, with congenital heart defects. The responsible gene, TBX5, belongs to the T-box family of transcription factors, which are crucial for, among other functions, mammalian limb development and identity (Basson et al. 1997). Interestingly, Okihiro syndrome (MIM 607343), which combines features of TBS and HOS, has recently been linked to mutations in SALL4, a homolog of SALL1, raising questions as to an interaction of TBX5 and SALL transcription factors (Kohlhase et al. 2002). Congruent with the localization of the human malformations, Tbx5 is expressed exclusively within the murine forelimb, whereas the related Tbx4 is constricted to the hindlimb. It is tempting to speculate that the T-box code has a function similar to that of the Hox code, in that it determines body-part identity. Tbx5−/− mice die in utero because of severe heart malformation before limb development is sufficiently advanced to assess limb identity. Human mutations are known that predominantly cause cardiac defects. For some of these, a differential impairment of binding to cardiac-specific promoters has been demonstrated (Basson et al. 1999).

Loss-of-function mutations in TBX3, which is active in the forelimb and hindlimb, are known to cause the dominant ulnar-mammary syndrome (MIM 181450) (Damy et al. 1997). The limb malformations affect primarily posterior structures—that is, the third to fifth digits and the ulna, which may be hypoplastic or deformed. A postaxial polydactyly can be also observed, and the fourth and fifth toe are frequently shortened. Tbx3 has been shown to be expressed in an anterior and a posterior stripe in the limb bud. The posterior stripe, which seemingly confines identity of the posterior digits, appears to be independent of ZPA signaling, whereas the anterior expression domain is sensitive to Bmp2 and Shh and is thought to determine the width of the bud (Tumpel et al. 2002).

Acheiropodia (MIM 200500) is a rare recessive condition with severe truncation defects of the upper and lower extremities and aplasia of the hands and feet. The limb phenotype somewhat resembles that of mice with a deletion of the Lmbr1 gene. The disorder can be identified in mice with a polyalanine expansion and a missense mutation (Goodman et al. 1998). The associated nephropathy is thought to be due to an altered expression of type IV collagen at the glomerular basement membrane, leading to defects in podocyte differentiation. Lmxb1b was shown to regulate the expression of this collagen (Dreyer et al. 2000). The pathomechanism underlying MPS appears to be haploinsufficiency (Dreyer et al. 2000).

Disorders Involving HOX Genes

The first human disorder discovered to be caused by a HOX gene mutation was synpolydactyly (SPD [MIM 186000]). Its hallmark is a dominantly inherited synpolydactyly between the third and fourth finger and the fourth and fifth toe. Additional features include clinodactyly, camptodactyly, and brachydactyly of the fifth finger, as well as syndactyly and brachydactyly of the second to the fifth toes. The genetic cause is an expansion of an imperfect polyalanine coding repeat in exon 1 of HOXD13 (Muragaki et al. 1996). The normally occurring 15 alanine residues in the N-terminal part of HOXD13 are expanded by 7–14 alanines in affected cases. In contrast to other repeat-expansion diseases, the repeat number is meiotically stable across generations. Repeat length positively correlates with severity and penetrance of the disorder (Goodman et al. 1997). Individuals in whom the longest repeat, 29 residues in total, was identified show additional malformations of the thumb and hallux and extraskeletal defects like hypoplasia. An even more pronounced phenotype, with severe brachydactyly and oligodactyly of the feet, was observed in individuals who are homozygous for the mutation (Muragaki et al. 1996). A much weaker phenotype results from deletion mutations that lead to frameshift and truncation of the protein or a missense mutation of a conserved residue (Goodman et al. 1998). Only few mutation carriers have the typical SPD features; instead, the second metatarsals can be duplicated, and the middle phalanges of the toes are slightly hypoplastic.

Interestingly, mice with inactivated Hoxd13 alleles show a very mild phenotype without polydactyly (Dolle et al. 1993; Davis and Capecci 1996), indicating that the SPD mutations are not simple loss-of-function mutations. The synpolydactyly homologue (spdh) mouse mutant has the SPD phenotype and a polyalanine expansion similar to the human mutation (Bruneau et al. 2001). The spdh phenotype closely resembles that of mice with deletions of the Hoxd11, Hoxd12, and Hoxd13 genes (Zakany and Duboule 1996), raising the possibility that the alanine expansions interfere with other limb-expressed
**Hox** genes. Further interesting findings are that, in *spdb* homozygotes, the proliferation of the autopod chondrocytes is reduced and that 5′ **Hox** genes are expressed in the perichondrium after birth. In addition to patterning, **Hox** genes apparently have a function in the regulation of growth (Albrecht et al. 2002).

**Hoxa13** is expressed in the distal limb bud, similar to **Hoxd13**. Heterozygous mutations in the **HOXA13** gene are the cause of hand-foot-genital syndrome (MIM 140000), a condition characterized by short first metacarpals, hypoplastic distal phalanges of the thumb and toes, and urogenital abnormalities such as duplications of the female urogenital tract, malpositioning of the ureteral orifices, and hypoplasias of varying degrees in affected males (Mortlock and Innis 1997). Most disease-causing mutations identified so far are nonsense mutations, which can be assumed to lead to a complete loss of function. Interestingly, an expansion of the polyalanine stretch at the N-terminus leads to an identical phenotype (Utsch et al. 2002). Hence, alanine expansions in **Hoxa13** produce loss of function, whereas similar expansions in the related **HOXD13** have complex effects.

Mutations in **HOXA11** have recently been shown to cause amegakaryocytic thrombocytopenia and radioulnar synostosis (MIM 605432) (Thompson and Nguyen 2000). The site of the malformation reflects the developmental expression of **HOXA11**. The mutations are single-base-pair deletions that are thought to cause a premature stop codon by frameshift, thus truncating the protein within the homeodomain. A loss of function is therefore likely.

**Disorders of Early Differentiation**

Pattern formation is the process during which number, size, and shape of the cartilaginous template are delineated. Once the pattern is determined, cells migrate to the sites of future skeletogenesis and form condensations that anticipate the structure of the skeleton (anlage). Extracellular matrix molecules such as versican, tenascin, syndecan, heparan sulfate, and chondroitin sulfate proteoglycans are highly expressed in these cells and contribute to the “sticky” nature of the cell aggregates. The next step is the overt differentiation of these cells into cartilage-forming chondrocytes in endochondral skeletal elements or into bone-forming osteoblasts in membranous skeletal elements.

In areas of endochondral bone formation, the condensed cells differentiate into chondrocytes. The transcription factor **Sox9** has been shown to be essential for this process (Bi et al. 1999). **Sox9** is a member of the Sox family of transcription factors, which are characterized by a high-mobility group–box DNA-binding domain. It is expressed in the early cartilaginous condensations and, at later stages, in growth-plate chondrocytes. One of its regulatory targets is **Col2a1**, the gene encoding the major collagen in cartilage; **Sox9** binds specifically to sequences within the first intron of this gene (Lefebvre et al. 1998). Two other members of the Sox family, **Sox5** and **Sox6**, are also required for overt chondrocyte differentiation. Homozygous **Sox5**/− mutant mice and **Sox6**/− mutant mice are born with relatively mild skeletal anomalies. In contrast, **Sox5/Sox6** double homozygous mice die in utero because of severe defects in cartilage formation. A detailed analysis has shown that **Sox9** is needed for the formation of mesenchymal condensations, whereas **Sox5** and **Sox6** are required for the differentiation of condensed cells into chondrocytes (Akiyama et al. 2002).

The transforming growth factor β (Tgfβ)/bone morphogenetic protein (BMP)/GDF5 pathway plays an important role in the regulation of condensation and differentiation of precursor cells into chondrocytes. Signaling of the Tgfβ superfamily members requires the binding of the ligand to cell-surface receptors consisting of two types of transmembrane serine/threonine kinase receptors, classified as type I and type II. The type II receptor transphosphorylates and thus activates the type I receptor. The intracellular substrates of the activated type I receptors are the Smads. Smads 1, 5, and 8 are phosphorylated and then translocated to the nucleus, where they participate in the transcriptional regulation of genes involved in cartilage and bone formation (Massague and Chen 2000; Miyazono et al. 2001). Overexpression of Bmp2 or Bmp4 in chick embryos is followed by dramatic increases in both the size and shape of skeletal elements. Similar effects can be observed by overexpressing a constitutively active BmpRlb, whereas the opposite effect is achieved by expressing a dominant negative truncated form of the receptor (Zou et al. 1997). The likely mechanism is the recruitment of mesenchymal precursor cells to the cartilage condensations and to the perichondria, which contribute cells to the anlage by appositional growth. Recruitment of cells to condensations is also consistent with the known action of BMPs and Gdf5 to induce cartilage and bone when implanted ectopically.

In areas of membranous bone formation, the condensed cells differentiate into osteoblasts, which produce bone matrix. Genetic experiments in mice have demonstrated that Chfα1/Runx2 is essential for this process (Otto et al. 1997). Runx2 is a member of a small family of transcription factors that are homologous to the *Drosophila runt* gene. In Runx2 null mice, no endochondral or membranous bone is formed, because of an arrest during the early steps of osteoblast differentiation. Recently, a Chfb-deficient mouse model with a similar yet less severe phenotype made clear that Runx2 directly interacts with Chfb to fulfill its role. A direct
interaction of both proteins was shown (Kundu et al. 2002). Osterix, another transcription factor, is thought to act downstream of Runx2 (Nakashima et al. 2002).

Mesenchymal condensations resemble the future skeleton in shape and size. In many instances, though, more than one bone or cartilage can arise from a single condensation. This is particularly evident in the limbs. The digital rays are thought to be formed from prechondrogenic condensations that appear spatially continuous and subsequently segment into individual skeletal elements. Morphologically, development of most joints begins with a condensation of cells at the future joints and the repression of chondrogenesis at these sites. In the chick, this so-called “interzone” develops into a three-layered structure, with the central region undergoing apoptosis resulting in the formation of the joint cavity. Recently, a member from the Wnt family, Wnt14a, has been implicated in joint induction (Hartmann and Tabin 2001). Members of the BMP/GDF families are expressed in the joint interspace and have also been reported to be involved in joint formation (Francis-West et al. 1999). Among them, Gdf5 is the most prominent. Inactivation of Gdf5, as observed in the brachydrathy types, results in the loss of interphalangeal joints. Multiple other genes involved in the regulation of chondrogenesis can be expected to play a role in this process. Overexpression of Runx2, for example, which is normally not expressed in the joint region, results in the lack of a morphologically defined interzone and missing Gdf5 expression, indicating that it interacts with the early events of joint formation (Stricker et al. 2002).

Genetic defects that result in the disturbance of mesenchymal condensation and/or differentiation can be expected to have a patterning-like phenotype—that is, loss or underdevelopment of certain bones, together with a growth defect. Such conditions have been termed “dysostoplasias,” indicating that aspects of dysostosis occur together with those of the dysplasias (Mundlos and Olsen 2002). Conditions that fit into this category can be found among the brachydactylies and other syndromes affecting early bone development.

Brachydactyly Types A, B, and C

Brachydactyly (from the Greek brachys, meaning “short,” and daktulos, meaning “digit”) is “shortening of the digits due to anomalous development of any of the contributing phalanges or metacarpals of which they consist” (Bell 1951, p. 2). Brachydactylies have been classified, on anatomic and genetic bases, into five groups, A to E, including three subgroups (A1, A2, A3) that usually manifest as autosomal dominant traits. Brachydactyly type A1 (BDA1 [MIM 112500]) is characterized by hypoplastic/aplastic middle phalanges. Interestingly, BDA1 was the first human condition recognized to be inherited in a dominant fashion. Named after this landmark paper, it is also called “Farabee-type brachydactyly.” BDA1 was recently shown to be caused by heterozygous missense mutations in the amino-terminal domain of the signaling molecule Indian hedgehog (IHH) (Gao et al. 2001). The role of Ihh during early digit formation is not well understood, and mice with one inactivated Ihh allele do not show a brachydactyly phenotype. Homozygous inactivation results in a severe and lethal chondrodysplasia characterized by the absence of cortical bone, reduced proliferation and abnormal differentiation of chondrocytes, and defects in joint formation (St-Jacques et al. 1999). The pivotal role of this signaling molecule in chondrocyte differentiation is discussed in the “Disorders of Growth” section.

Brachydactyly type B (BDB [MIM 113000]) is characterized by hypoplasia/aplasia of the distal phalanges and/or nails. The thumb is usually unaffected but may be duplicated in severe cases. Symphalangism, especially of the interdigital joints, occurs. BDB is caused by truncating mutations located in two distinct regions of the receptor tyrosine kinase ROR2 (Oldridge et al. 2000). The truncations distal to the tyrosine kinase domain are associated with a more severe phenotype than are proximal mutations, which disrupt or delete this entire domain (Schwabe et al. 2000). Ror2 has a pivotal role in chondrocyte differentiation, as demonstrated by the severe and lethal chondrodysplasia observed in Ror2 null mice (Takeuchi et al. 2000). However, the mechanism explaining the brachydactyly phenotype remains unclear.

Brachydactyly type C (BDC [MIM 113100]) is characterized by brachymesophalangy of the second, third, and fifth fingers, sometimes together with hyperphalangy, usually of the second and third fingers, and shortening of the first metacarpal. As a rule, the fourth finger is not affected and is therefore the longest digit in BDC. Heterozygous frameshift or nonsense mutations affecting GDF5, also called “cartilage-derived morphogenetic protein 1” (CDMP1), have been identified in several individuals with BDC (Polinkovsky et al. 1997). Homozygous mutations located within the active domain of GDF5 lead to severe disturbance of limb morphogenesis in acromesomelic chondrodysplasias of the Grebe (MIM 200700) (Thomas et al. 1997) and Hunter-Thompson (MIM 201250) (Thomas et al. 1996) types, as well as Du Pan syndrome (MIM 228900) (Faiyaz-Ul-Haque et al. 2002).

Defects of Joint Formation

Abnormalities in joint formation occur in several genetic conditions. Patients with proximal symphalangism (MIM 185800) show fusion/malformation of the prox-
imal interdigital joints together with deafness due to an ankylosis of the stapes within the middle ear. Multiple-synostosis syndrome (MIM 186500), a more severe form of this condition, was later shown to be allelic. Heterozygous mutations affecting the Bmp antagonist noggin (NOG) (Gong et al. 1999; Marcelino et al. 2001) were shown to be the cause of these conditions.

*Campomelic Dysplasia*

Campomelic dysplasia (MIM 114290) is a dominant disorder caused by haploinsufficiency of the transcription factor SOX9 (Wagner et al. 1994). The phenotypic abnormalities include (i) bowing and angulation of long bones, (ii) hypoplasia of the scapula and pelvis, (iii) abnormalities of the vertebral column with a decreased number of ribs, and (iv) craniofacial abnormalities (cleft palate, micrognathia, hypertelorism, and a flat facies) (fig. 3). Most affected babies die, in the neonatal period or during early infancy, of respiratory distress caused by a small rib cage, narrow airways (defective tracheobronchial cartilage), and hypoplastic lungs. However, long-term survivors have been described. Three-quarters of the cases with a male karyotype have a complete or partial sex reversal. Other tissues affected include kidney, heart, brain, and pancreas, consistent with the expression pattern of Sox9 in the developing mouse.

Four major classes of mutations causing campomelic dysplasia have been reported. The missense mutations impair DNA binding, a finding that is consistent with a loss-of-function model. The truncating mutations reduce transactivation activity and thereby impede the ability of SOX9 to activate target genes. SOX9 has a large 5’ regulatory region, and translocations or deletions in this area apparently interfere with normal SOX regulation (Meyer et al. 1997).

*Ellis–van Creveld Syndrome*

Ellis–van Creveld syndrome (MIM 225500) is also referred to as “chondroectodermal dysplasia.” It is a recessive disorder, and—as the designation implies—the clinical features are a combination of skeletal and ectodermal abnormalities. The major features consist of (i) short stature, of prenatal onset, that is most pronounced in the distal segments; (ii) skeletal defects, including polydactyly, short and broad middle phalanges and hypoplastic distal phalanges, fusion of wrist bones, an abnormal pelvis, a narrow thorax with thin ribs, and hypoplasia of the upper lateral tibia; (iii) hypoplastic fingernails; (iv) cardiac defects; (v) neonatal teeth, or small teeth with delayed eruption; and (vi) a frenulum from the alveolar ridge to the upper lip, as well as defects in the alveolar ridge. The responsible gene, called EVC, was mapped in an Amish pedigree and encodes a 992–amino acid protein of unknown function containing a leucine zipper motif and nuclear localization signals (Ruiz-Perez et al. 2000).

*Cleidocranial Dysplasia*

Cleidocranial dysplasia (CCD [MIM 119600]) is a dominantly inherited disorder caused by mutations in CBFA1/RUNX2 (Mundlos et al. 1997). CCD is characterized by (i) hypoplasia/aplasia of the clavicles; (ii) delayed ossification of cranial sutures and fontanelles; (iii) dental anomalies, including delayed eruption of deciduous and permanent teeth and supernumerary teeth of the permanent dentition; and (iv) short stature. A number of other skeletal abnormalities are commonly observed, including a narrow, bell-shaped thorax; cervical or missing ribs; and characteristic changes in the pelvis, consisting of hypoplastic iliac wings and a wide symphysis pubis. Large femoral epiphyses, two ossification centers of the second metacarpal, and a short middle phalanx of the fifth finger are frequently found. The bone development is generally retarded. The face and the skull have a characteristic appearance, with a relatively large cranium, frontal and parietal bossing, and a frontal groove originating from ossification defects.
within the metopic suture. The nose is short and antverted, and the maxilla is hypoplastic, giving the impression of a relative prognathism (Mundlos 1999).

CCD is caused by haploinsufficiency of Runx2. The mutations identified so far include deletions of the entire gene and flanking regions; nonsense mutations and frame shifts, which can be expected to result in RNA degradation; and missense mutations in the DNA binding domain, which were shown to interfere with DNA binding (Quack et al. 1999; Otto et al. 2002). Interestingly, some of the truncating mutations that may result in stable proteins were shown to delete binding sites for Smad. A missense mutation affecting serine 104 was shown to interfere with heterodimerization of Runx2, thereby mimicking the inhibitory effect of phosphorylation of this residue (Wee et al. 2002).

CCD is a typical condition that combines features of a dysostosis—that is, abnormal patterning (aplasia of clavicles and supernumerary teeth)—with those of a skeletal dysplasia affecting the skeleton as a whole (short stature). This combination of defects can be explained by the dual role of Runx2 in skeletal development. First, it is essential for the differentiation of osteoblasts by tightly controlling a number of downstream genes. This control appears to be exquisitely dose sensitive, since even a 50% reduction is sufficient to produce a phenotype. Runx2\(^{-/-}\) mice are a good model for CCD, since they recapitulate most of the human phenotype (with the exception of supernumerary teeth) (Otto et al. 1997). The skull phenotype can be explained by a reduced rate of differentiation of precursor cells into osteoblasts at the site of bone growth—that is, the suture lines. The clavicles are hypoplastic, with delayed endochondral ossification of the medial part and absent intramembranous formation of the lateral part (Huang et al. 1997). A second role for Runx2 is in the regulation of chondrocyte hypertrophy and the invasion process, which explains the delay in bone maturation and the short stature observed in patients with CCD (Kim et al. 1999).

Furthermore, Runx2 appears to have a role beyond embryonic development. Some individuals with CCD have very low bone density and suffer from osteoporotic fractures (Quack et al. 1999; Morava et al. 2002; Unger et al. 2002). Whether this is caused by the downregulation of downstream genes such as alkaline phosphatase or by a reduced osteoblast differentiation remains to be determined.

**Leri-Weill Dyschondrosteosis and Langer Mesomelic Dysplasia**

Leri-Weill dyschondrosteosis (LWD [MIM 127300]) and Langer mesomelic dysplasia (MIM 249700) are caused by mutations in the short stature homeobox gene (SHOX) (Belin et al. 1998; Stuppia et al. 1999; Clement-Jones et al. 2000; Zinn et al. 2002). It resides within the pseudoautosomal regions on the X and Y chromosomes and may also play a role in nonsyndromic short stature and in Turner syndrome. LWD is caused by haploinsufficiency, whereas Langer mesomelic dysplasia is due to homozygous loss-of-function mutations. LWD is characterized by short stature, Madelung deformity of the forearm, and limited motion at the elbow. Langer mesomelic dysplasia results in a rudimentary fibula, a very short dysplastic tibia, a distally reduced ulna, and a bowed shortened radius. Additional features are short fingers, broad hands, and hypoplastic mandibles. The target genes and the exact role of SHOX are unknown. The combination of symptoms, however, suggests a role in early chondrocyte differentiation as well as in the growth plate.

**Disorders of Growth**

In contrast to the bones of the skull that are formed by a direct transformation of mesenchymal cells into osteoblasts, so-called “desmal ossification,” the major part of the skeleton is formed by endochondral ossification. In endochondral ossification, a cartilaginous template is formed first, which is subsequently replaced by bone. Central to this process is the formation of a growth plate, a highly organized structure that generates all of the longitudinal growth (fig. 4). Growth-plate chondrocytes are invariably arranged in three layers: reserve chondrocytes, proliferating chondrocytes, and hypertrophic chondrocytes. A very complex interplay of different signaling pathways regulates the rate of proliferation and the conversion of proliferating chondrocytes into hypertrophic chondrocytes. During the process of chondrocyte differentiation, the matrix composition changes dramatically through the production of other components, such as collagen type X; the expression of metalloproteinases; and calcification. At the same time, blood vessels begin to penetrate the calcified cartilage, bringing in osteoclasts, which remove cartilage, and osteoblasts, which build new bone. With further growth, the central and primary center of ossification expands toward the ends of the bones, and secondary centers of ossification form within the cartilage remnants. The growth plate, now localized between the epiphysis (the secondary center of ossification) and the metaphysis (the distal end of former primary ossification center), remains active until the end of puberty, when primary and secondary ossification centers fuse. At this point, the cartilage of the joints is the only cartilage that remains of the former anlage.

Growth hormone secreted by the pituitary gland and IGF1 produced by proliferating and hypertrophic chondrocytes, among many other tissues, act largely independently to control the rate of chondrocyte proliferation.
Disorders affecting this pathway result in proportionate dwarfism without much skeletal dysplasia, suggesting that they are major regulators of linear bone growth and, thus, of body size. The vast majority of other short-stature disorders, however, result in disproportionate dwarfism, presumably because they affect not only proliferation but also differentiation of chondrocytes.

Two major signaling pathways that control proliferation and differentiation of chondrocytes have been identified: the Ihh/parathyroid hormone (PTH)–related peptide (PTHrP) pathway and the FGF pathway. Ihh, the second mammalian hedgehog ortholog, plays a central role in the regulation of chondrocyte proliferation and hypertrophy. Ihh signals through the HH pathway (also see the “Disorders of Skeletal Patterning” section). Ihh appears to have direct and indirect functions. Through a yet-unknown cascade of events, Ihh indirectly regulates the expression of PTHrP, which is normally expressed in chondrocytes close to the joint surface. PTHrP is a secreted molecule transducing its signal via the PTH/PTHrP receptor (PPR) and plays a critical role in the regulation of chondrocyte hypertrophy by keeping cells in the nonhypertrophic proliferative state. Ihh and PTHrP form a feedback loop whereby Ihh up-regulates the synthesis of PTHrP, thereby indirectly slowing down the process of chondrocyte hypertrophy (Vortkamp et al. 1996).

FGF signaling plays a major role in the regulation of chondrocyte proliferation and differentiation. More than 20 different FGFs are currently known, and splice variants further widen the diversity. They are involved in a multitude of developmental and physiological processes, act as potent mitogens for chondrocytes as well as for osteoblasts, and stimulate bone formation in vitro and in vivo. The four distinct FGF receptors (FGFRs) are activated by most members of the FGF family. Activity and specificity of the FGF/FGFR complex is dependent on the tissue-specific expression of splice variants and the composition of the extracellular matrix (ECM), which binds FGFs and is an indispensable cofactor for activa-
tion of the FGF receptors. Only few downstream targets of FGFs have been elucidated, among them PLCγ, Shc, MAP kinase, and the transcription factor STAT1. FGFR1 and FGFR3 are both expressed in the growth plate. Their distinct domains of expression (FGFR3 is expressed in proliferating chondrocytes, whereas FGFR1 is expressed in prehypertrophic and hypertrophic chondrocytes) suggest unique functions. Inactivation of Fgr3 in the mouse results in overgrowth of the long bones, whereas expression of an activating mutation results in dwarfism, indicating that Fgr3 functions as a regulator of chondrocyte proliferation and/or hypertrophy (for a review of FGF function see Ornitz and Marie 2002). It is not yet clear which of the many different FGFs expressed in the developing endochondral bone is the predominant ligand for FGFR3 at the growth plate. However, the similarity between the phenotype of a recently created Fgf18 knockout and the Fgr3-deficient mouse model indicates that it may be Fgf18 that rules the growth plate in competition with its opponents, the BMPs (Liu et al. 2002).

The Ihh/PTHrP and the FGF signaling pathways also interact with members of the BMP family. At the growth plate, FGFs have been shown to antagonize BMP action by inhibiting Ihh expression and to decrease chondrocyte proliferation by accelerating chondrocyte hypertrophy (Minina et al. 2002).

The next and final step in endochondral bone formation is the replacement of hypertrophic cartilage by bone. This requires vascular invasion, a step initiated and controlled by an interaction of antiangiogenic and angiogenic factors. To stimulate vessel invasion from the bone collar, the hypertrophic chondrocytes express vascular endothelial growth factor (VEGF). As pointed out in the “Disorders of Early Differentiation” section, Runx2 is a transcription factor of pivotal importance for bone formation. In cartilage, Runx2 is expressed in nonproliferative and prehypertrophic chondrocytes (Stricker et al. 2002), one of its target genes being VEGF (Zelzer et al. 2001). If Runx2 is expressed specifically in nonhypertrophic chondrocytes in Runx2<sup>−/−</sup> mice, it is able to partially rescue the differentiation defect and induces chondrocyte hypertrophy and endochondral ossification (Takeda et al. 2001). Furthermore, despite the absence of osteoblasts in rescued animals, osteoclasts resorbing the cartilage matrix were identified. Hence, Runx2 seems to induce chondrocyte hypertrophy and the consecutive removal of calcified cartilage in parallel with its key function in osteoblast differentiation. VEGF is not secreted by the hypertrophic chondrocytes, but it is walled in the hypertrophic cartilage. As a fascinating regulatory mechanism, it was shown to be released by the action of matrix-metalloproteinase-9 (MMP-9), which is produced by osteoclasts (Vu et al. 1998). The hypertrophic chondrocytes furthermore ensure their posthumous removal by expressing MMP-13, which alone has little proteolytic activity but is strongly activated by MMP-9 (Engsig et al. 2000).

Cartilage is characterized by a unique ECM that accounts for ~90% of the tissue volume. The main component is fibrous collagen, which confers tensile strength. In the resting and proliferating chondrocytes, production of type II collagen predominates, which can form fibers together with type IX and type XI collagen. Type X collagen, in contrast, is specific to hypertrophic cartilage. Proteoglycans—above all, aggregan—are of high abundance. They are giant molecules with a gel-like consistency that are composed of a core protein to which are attached different kinds of glycosaminoglycans (GAGs), which are highly sulfated and thus negatively charged. This allows them to bind large amounts of cations and water molecules and to be mutually repellant, which is thought to contribute to the elasticity of the cartilage. Glycoproteins are a third group of matrix components, comprising, among others, perlecain, fibronectin, tenascin, and cartilage oligomeric matrix protein (COMP). COMP is a pentameric glycoprotein able to bind calcium at calmodulin-like domains. It forms noncovalent cross-links by interacting with different types of collagens.

Bone consists mostly of type I collagen. In contrast to the elastic cartilage, the bone matrix contains few proteoglycans and instead consists of as much as two-thirds hydroxyapatite, in order to be rigid.

Disorders Affecting Chondrocyte Differentiation and Proliferation

Mutations in IHH, one of the key regulators of chondrocyte proliferation and differentiation, cause BDA1, as described in the “Disorders of Early Differentiation” section. In addition to hypoplasia/aplasia of the middle phalanges, BDA1 is, in some individuals, associated with short stature, confirming its general role in growth-plate function (Gao et al. 2001). Ihh is a secreted signaling molecule that exerts its function over long distances. The composition of the ECM appears to be of utmost importance for this process, as is demonstrated by the Dro sophila mutant tout velu. In these animals, the distribution of hedgehog is disorganized by an altered expression of heparan sulfate (Bellalie et al. 1998). Loss-of-function mutations in the EXT genes, the human orthologs of tout velu, cause multiple cartilaginous exostoses (MIM 133700), a condition characterized by multiple, circumscribed, sometimes painful bone protuberances, most frequently located at the ends of tubular bones in close proximity to the growth plates. During adulthood, growth of the lesions slows, and no new lesions are formed. Additional dysplastic changes are disproportionate shortening of the ulna with bowing of the radius and radioulnar or tibiofibular synostosis. Adult height is frequently reduced.
Malignant degeneration occurs and is caused by second hits involving other genes. EXT1 is located on chromosome 8q24, EXT2 is located on chromosome 11p11-12, and other loci have also been described. Mutations in EXT1 cause multiple exostosis and, if involved in a contiguous deletion, are part of tricho-rhino-phalangeal syndrome (MIM 190350) (Ludecke et al. 2001).

Several disorders have been described that involve PTHrP and the PPR. Blomstrand chondrodysplasia (MIM 215045) is characterized by a short-limb-type dwarfism, with severely shortened tubular bones with club-shaped ends, an accelerated bone maturation, shortened limbs, and a generalized sclerosis of bone. Affected children usually die shortly after birth. The early maturation of the skeleton is caused by an unhindered chondrocyte hypertrophy due to homozygous loss-of-function mutations in the PPR (Jobert et al. 1998). Dominant activating mutations in the same receptor result in Jansen-type metaphyseal chondrodysplasia (MIM 156400) (Schipani et al. 1995). Patients suffer from a severe growth retardation and bone deformations. The joints are enlarged, with osseous restriction of mobility and ligamentous hyperlaxity. Radiologically, there is generalized demineralization (resulting in occasional fractures), rachitiform changes with enlargement of the metaphyses, and hyperostosis of the calvarium and the base of the skull. Major laboratory findings include hypercalcemia during the first months of life, elevated alkaline phosphatase, and low or normal serum PTH. Two different missense mutations lead to a constitutive stimulation of cAMP production by the receptor, which blocks chondrocyte hypertrophy and hinders the following ossification process. Enchondromatosis (MIM 166000) is characterized by the occurrence of multiple enchondromas, benign tumors of cartilage that are usually located in close proximity to or in continuity with growth plate cartilage. In some cases, a generalized dysplasia may be present that is not attributable to the enchondromas. Enchondromas can lead to skeletal deformity, and, in rare cases, may result in chondrosarcoma. A mutant PPR receptor has been identified in some cases, which constitutively activates hedgehog signaling. The mutations can be inherited as germline mutations that acquire second hits later in life or as somatic mutations that can be detected only within the lesion (Hopyan et al. 2002).

The importance of FGF signaling for skeletal growth first became clear when it was discovered that mutations in FGFR3 lead to achondroplasia (MIM 100800), the most frequent form of dwarfism (Shiang et al. 1994). Rhizomelic limb shortening, midface hypoplasia, frontal bossing, trident hands, and exaggerated lumbar lordosis are typical of this dominantly inherited condition. The disproportionately large head may be due to true megencephaly, or, more commonly, it may be due to enlarged ventricles that are believed to be caused by increased intracranial pressure due to diminished resorption and drainage of cerebrospinal fluid. The hydrocephalus stabilizes spontaneously in almost all patients (achondroplasia-specific growth curves for head circumference should therefore be used) and is not related to intellectual performance, which is normal in individuals with achondroplasia. Muscular hypotonia is commonly found in infancy and may be caused by stenosis of the foramen magnum. Guidelines for health supervision of children with achondroplasia can be found in the 1995 American Academy of Pediatrics (AAP) Guidelines (see the “Health Supervision for Children with Achondroplasia” policy statement, available from the AAP Web site). Mean ± SD adult height is 131 ± 5 cm in males and 124 ± 6 cm in females. Two allelic disorders exist: Hypochondroplasia (MIM 146000) is a milder form of the disorder, with less growth deficit and more or less normal facial development. Thanatophoric dysplasia (MIM 187600 and MIM 187601), in contrast, is characterized by severe disproportionate dwarfism with very short extremities, very narrow thorax, and death in early infancy due to respiratory insufficiency. Major radiographic features include severe platyspondyly, short and relatively broad tubular bones and bowing of the femora, small facial bones and large calvaria, and distinct changes of the pelvis. Two types are differentiated, with type 2 showing straight femora and craniosynostosis.

All the FGFR3 mutations involved in the three conditions have a receptor-activating effect, thus leading to ligand-independent tyrosine phosphorylation. The mutations, however, lie in very different regions of the protein. The most common achondroplasia mutation (G380R) is located in the transmembrane segment, whereas hypochondroplasia is most often caused by the N540K mutation, which modifies a tyrosine kinase domain (Shiang et al. 1994; Bellus et al. 1995). Thanatophoric dysplasia type 1 is caused, in the great majority of cases, by an extracellular R248C mutation, whereas type 2 is generally due to a K650E substitution within the tyrosine kinase domain of the receptor. For at least some of these mutations, a correlation between receptor tyrosine kinase activity and severity of the phenotype could be shown (Naski et al. 1996). Interestingly, another FGFR3 mutation (P250R) in the extracellular domain was identified in several craniosynostosis cases (Bellus et al. 1996).

The broad spectrum of FGF action is demonstrated by autosomal dominant hypophosphatemic rickets (MIM 605380), the only heritable disorder linked to mutations in an FGF so far. The common rickets phenotype, which is characterized by a growth defect due to growth-plate disorganization and mineralization defects, is caused by a calcium deficit, which leads to an increase of PTH secretion. In hypophosphatemic rickets, however, PTH levels are normal. In contrast, this phenotype is caused by
an accumulation of FGF23 by mutations, which make it resistant to inactivation by peptidase cleavage (The ADHR Consortium 2000). Nearly identical symptoms are produced by inactivating mutations in the metallopeptidase gene *PHEX* in X-linked hypophosphatemia (The HYP Consortium 1995). Recent results suggest that FGF23 is cleaved by PHEX, which explains the close relationship between both forms of rickets (Bowe et al. 2001). Whereas the exact function of FGF23 in growth plate chondrocytes and bone cells is only beginning to be unraveled, it was clearly shown that it downregulates the sodium-phosphate cotransport in kidney cells, thus causing the hypophosphatemia of the two disorders.

Another receptor tyrosine kinase implicated in growth regulation is ROR2, the receptor tyrosine kinase mentioned above in connection with BDB. A mouse model lacking Ror2 shows small but normally shaped limb anlagen at early developmental stages but develops misshapen and shortened limbs because of dysregulated enchondral ossification. (DeChiara et al. 2000). The murine phenotype resembles human recessive Robinow syndrome (MIM 268310), a skeletal dysplasia with (i) characteristic facial appearance, (ii) chondrodysplasia with short stature and mesomorphic shortening of the limbs, (iii) hypoplasia of the genitals, (iv) vertebral malformations, and (v) heart defects. In contrast to BDB, in which two mutational hotspots are located just before or after the tyrosine kinase coding domain, the mutations in Robinow syndrome are spread throughout the gene and are expected to result in a loss of function (Afzal et al. 2000).

**Disorders Caused by Defects in Matrix Components**

ECM is essential for proper functioning of cartilage and bone. Most abundant are the collagens, which are homomeric or heteromeric triple helices of three collagen chains. Type II collagen is a homomer of three \( \alpha_1 \) chains encoded by the *COL2A1* gene. A spectrum of different dominant disorders is linked to this gene, the most deleterious being achondrogenesis type II (MIM 200610). This condition is characterized by (i) very short limbs; (ii) flat midface, micrognathia, and often cleft palate; and (iii) hydropic appearance. Radiographically, the absent or severely retarded ossification of the vertebrae, barrel-shaped thorax, and very short tubular bones with metaphyseal flare and cupping are most striking. Hypochondrogenesis (MIM 200610) is part of the spectrum and shows similar but less severe changes. The cause of both conditions is missense mutations at the C-terminal end of the helix-forming part of the collagen protein (Chan et al. 1995). Collagen containing mutant molecules is posttranslationally overmodified and not secreted, resulting in the complete absence of type II collagen from the cartilage matrix (Mundlos et al. 1996).

Spondyloepiphyseal dysplasia congenita (SEDc [MIM 183900]) and Kniest dysplasia (MIM 156550) are milder forms, the first being caused by missense mutations in the C-terminal half of the helix-forming region and the latter by splice-site mutations, which lead to small, nontruncating deletions (Spranger et al. 1994). Both conditions are characterized by disproportionate short-spine dwarfism with normal-sized hands and feet, a flat midface, occasional cleft palate, and severe myopia in \(~50\%\) of cases. Retinal detachment is a common complication. Stickler dysplasia (MIM 120140) represents the mild end of the collagen type II–related disease spectrum. Major clinical findings are (i) midface hypoplasia; (ii) cleft palate and Pierre-Robin sequence in infants; (iii) a high degree of myopia; (iv) sensorineural hearing loss; (v) normal stature or only mild shortness of stature, sometimes with Marfanoid appearance; and (vi) joint pain, morning stiffness, and precocious arthritis. In contrast to the more severe forms, Stickler dysplasia is generally caused by nonsense mutations, resulting in a nonfunctional allele, or by mutations in the N-terminal half of the collagen helix-forming region, preventing the insertion of the molecule into the type II collagen trimer (Winterpacht et al. 1993).

Collagen type II forms fibrils with other collagens—in particular, collagen type XI. This biochemical finding is mirrored on a genetic basis by mutations in *COL11A1* and *COL11A2*, the two genes encoding the \( \alpha_1 \) and \( \alpha_2 \) chains of collagen type XI, in Stickler dysplasia. Other conditions caused by mutations in collagen type XI are the recessive and dominant variants of otospondylo-megaepiphyseal dysplasia (MIM 215150) (Vikkula et al. 1995).

Defective matrix components are also responsible for the different types of multiple epiphyseal dysplasias (EDMs) (Briggs and Chapman 2002). Usually beginning at age 2–10 years, typical symptoms are pain and stiffness in the joints, the knee and the hip being most heavily affected. Osteoarthritis develops in early adulthood. Body height is normal or sometimes slightly reduced. Fragmentation and delayed ossification of numerous epiphyses are visible radiographically. The genes underlying EDM2 (MIM 600204) and EDM3 (MIM 600969) are *COL9A2* and *COL9A3*, respectively. A recently reported EDM mutation in *COL9A1* completed this list (Czarny-Ratajczak et al. 2001). All EDM mutations affect splice sites, resulting in mRNAs bearing in-frame deletions. The cartilage collagen mainly consists of type II and type XI fibrils. Type IX collagen is restricted to the surface of the mature collagen fiber, which possibly explains the relatively mild phenotype.

A more severe form of EDM (EDM1 [MIM 132400]) and pseudoachondroplasia (MIM 177170), a condition characterized by a short-limb dwarfism with body proportions resembling those of achondroplasia but with a
normal face, are caused by mutations in cartilage oligomeric matrix protein (COMP). Most mutations affect the calmodulin-like domains, leading to retention of the misfolded protein in the rough endoplasmatic reticulum. Since COMP interacts with type I, II, III, and IX collagen, these are also retained within the chondrocytes, leading to cell death (Thur et al. 2001). Another gene product associated with EDM (EDM5 [MIM 607078]) is matrilin-3 (MATN3) (Chapman et al. 2001). MATN3 is a matrix protein carrying von Willebrand factor A domains that appear to interact with COMP and with type II and type IX collagen (Briggs and Chapman 2002).

A recessive form of EDM (EDM4 [MIM 226900]) shows clubfoot, scoliosis, and a double-layered patella as additional features. Mutations in the diastrophic dysplasia sulfate transporter (DTDST) are underlying this disorder (Superti-Furga et al. 1996b). Similar to the type II collagenopathies, a spectrum of diseases are associated with mutations in DTDST (Rossi and Superti-Furga 2001). Diastrophic dysplasia (MIM 222600), the disorder in which the transporter was first identified, is a recessive dwarfing condition with a high frequency in Finland (Hastbacka et al. 1994). This condition is characterized by (i) multiple joint contractures resulting in therapy-resistant club feet; (ii) proximally set, hypermobile thumbs; (iii) cystic masses of the external ear (cauliflower appearance); (iv) cleft palate; and (v) scoliosis, which is progressive in most cases. Atelosteogenesis type 2 (MIM 256050) presents with the same pattern of abnormalities as diastrophic dysplasia but is more severe. Achondrogenesis type 1B (MIM 600972) represents the most severe end of the DTDST spectrum, showing hydropic appearance, extreme micromelia, short trunk, absent to severely retarded ossification, and, most characteristically, extremely short tubular bones with loss of longitudinal orientation and round or triangular configuration (Superti-Furga et al. 1996a). As mentioned above, glycosaminoglycans are not only major matrix components, but, like heparan sulfate, also important cofactors for FGF signaling. Inactivation of the sulfate transporter DTDST results in the production of undersulfated cartilage proteoglycans disturbing growth regulation as well as matrix integrity. EDM is mostly caused by missense mutations, as is diastrophic dysplasia. In contrast, stop mutations or substitutions in transmembrane segments lead to the more severe phenotypes. A rough correlation between the reduction of sulfate transport and disease severity has been found when mutated DTDST is expressed in Xenopus oocytes (Karniski 2001).

Perlecan is a heparan sulfate proteoglycan present in all basement membranes and in cartilage (which does not have basement membranes). Perlecan is implicated in cell growth and differentiation through interactions with growth factors, cell surface receptors, and ECM molecules. Mice with an inactivated perlecan gene (\(Hspg2^{−/−}\)) develop severe chondrodysplasia (Arikawa-Hirasawa et al. 1999). Their phenotype somewhat resembles the lethal autosomal recessive disorder dyssegmental dysplasia, Silverman-Handmaker type (MIM 224410), which led to the identification of mutations in the human perlecan gene (Arikawa-Hirasawa et al. 2001). Dyssegmental dysplasia is a lethal condition with irregularly sized vertebral bodies that have single or multiple ossification centers, small thorax and ribs, and very short, extremely widened tubular bones. Patients with dyssegmental dysplasia of the Roland-Desbuquois type have similar but less severe changes. Phentypic overlaps with Kniest dysplasia exist. Both conditions are caused by insertions and splicing mutations in the perlecan gene HSPG2 and are predicted to result in unstable molecules that are not secreted. Furthermore, structural and functional mutations have been shown to cause Schwartz-Jampel syndrome (MIM 235800) (Nicole et al. 2000), a recessive skeletal dysplasia associated with myotonia. Patients with Schwartz-Jampel syndrome survive and show much milder phenotypes compared with the dyssegmental dysplasias, possibly reflecting differences in the amount of perlecan in the matrix.

The major component of bone matrix is collagen type I, which is encoded by two genes, \(COL1A1\) and \(COL1A2\). Mutations in these genes result in osteogenesis imperfecta (OI), or “brittle bone disease” (for review, see Byers and Cole 2002). OI covers a wide phenotypic spectrum of conditions characterized by osseous fragility and fractures. On clinical, genetic, and radiological grounds, four major subtypes have been delineated. The most severe cases, classified as the neonatal type or OI type II, have (i) a characteristic facial appearance with dark sclerae and a beaked nose, (ii) an extremely soft calvarium because of insufficient ossification, (iii) short extremities with bowed legs, and (iv) a small thoracic cavity. The radiological picture shows a virtual absence of calvarial mineralization, short and malformed (“crumpled”) long bones, beaded ribs, and signs of fractures with insufficient healing. Severe nonlethal cases of the progressive deforming variety are generally diagnosed as type III OI. Many of these patients eventually become wheelchair bound because of the recurrent fractures and poor healing resulting in severe deformity and instability of the skeleton. Patients with type IV OI show mild to moderate deformity, short stature, and dentinogenesis imperfecta. Type I refers to the least severe variety. Affected individuals have blue sclerae, normal teeth, and normal to near-normal stature, but they have recurrent fractures that heal without deformity. Very mild cases of OI may have osteopenia as the only clinical sign. The majority of cases with OI types II–IV are caused by mutations, in either \(COL1A1\) or \(COL1A2\), that replace one of the repetitive glycines within the triple-helical domain with other, bulkier amino acids. This disrupts the correct folding of the helix and thus has a
dominant effect by inactivating a high percentage of the normal chains as well. Similar to type II collagen, the less severe phenotype (i.e., type I OI) is caused by nonsense mutations resulting in mRNA degradation and by other mutations that result in silent alleles or the exclusion of the mutated protein from the helix. Multiple subtypes of OI with dominant and recessive inheritance that are not caused by mutations in either of the collagen type I genes have been described, expanding the Silence classification (Cole 2002). Recent studies have shown that severe cases of OI can be effectively treated with bisphosphonates, agents that interfere with bone resorption and decrease bone loss in postmenopausal woman with osteoporosis (Rauch et al. 2002). The treatment appears to selectively inhibit resorption, leaving bone formation intact.

Disorders of Skeletal Homeostasis

As soon as the first bone tissue is formed, osteoclasts/chondroclasts come into play and start to degrade calcified cartilage as well as mature bone. This resorptive activity is a prerequisite for bone growth, since formation of mature bone tissue is possible only after the removal of calcified cartilage. Proper growth and skeletal maturation is important to reach an adequate peak bone mass, which has to be kept stable as long as possible. On the other hand, it is pivotal that the bone architecture adjust constantly to changing mechanical and metabolic needs. Both preservation of overall bone mass and constant restructuring are achieved by a concerted action of osteoblasts and osteoclasts, called “bone remodeling.” If remodeling is out of balance, either a loss or an accumulation of bone occurs, resulting in osteoporotic or osteopetrotic phenotypes, respectively.

Bone Matrix and Mineralization

Bone consists mostly of type I collagen and hydroxyapatite. Osteonectin, bone sialoprotein, and tissue-non-specific alkaline phosphatase (TNAP), three of the several glycoproteins found in bone, are thought to regulate hydroxyapatite deposition. Osteoblasts first secrete osteoid that subsequently calcifies in a not completely understood manner. Hydroxyapatite crystal formation is inhibited by pyrophosphate (PPi), which is cleaved by TNAP to phosphate (P). This explains why TNAP-deficient mice have a delayed ossification and thus reduced bone growth. Extracellular PPi levels seem to be regulated by the Ank protein, which transports PPi. Osteocalcin has, despite what its name suggests, only a minor effect on bone mineralization but was shown to regulate cross-linking of other matrix proteins and to inhibit osteoblast function. It is one of the few proteins that are specifically transcribed by the osteoblast. Some noncollagenous matrix proteins such as vitronectin, osteopontin, and bone sialoprotein contain RGD motifs, which are recognized by integrins and mediate cell attachment. Furthermore, bone matrix proteins were shown to bind to growth factors—for example, Tgfβ. Hence, osteoblasts and osteoclasts can be regulated in different ways by interaction with the bone matrix—or can become dysregulated, in the case of a disturbance of the matrix components.

Osteoblast Differentiation

Desmal ossification and endochondral ossification are dependent on Runx2 activity. Without Runx2, mesenchymal precursor cells cannot differentiate into osteoblasts. Ihh controls chondrocyte differentiation but is also essential for osteoblast differentiation, as demonstrated by the total absence of osteoblasts in long bones of Ihh-deficient mice (St-Jacques et al. 1999). In Ihh−/− mice, the perichondrium, from which osteoblasts are recruited during the endochondral ossification process, lacks expression of Runx2, indicating that Ihh acts upstream of Runx2. FGF18 also stimulates osteoblast differentiation and proliferation. It was shown to be expressed in differentiating osteoblasts, and FGF18 deficiency causes delayed ossification and osteoblast differentiation (Liu et al. 2002).

Local and Systemic Factors Influence Osteoblast Proliferation and Function

LRP5 is a ubiquitously expressed coreceptor for Wnt growth factors that becomes upregulated during osteoblast differentiation and which is inhibited upon binding to proteins of the Dickkopf (dkk) family. In vitro experiments and data obtained from the analysis of knockout mice suggest that it regulates osteoblast proliferation and function via the canonical pathway involving β-catenin, which is independent of Runx2 (Kato et al. 2002).

Different local factors primarily regulate osteoblast proliferation and activity. Although BMPs stimulate the expression of Runx2 in osteoblasts in vitro, their role in skeletal homeostasis has remained ambiguous, since their inactivation primarily results in abnormal patterning. BMP3-deficient mice, however, have a phenotype compatible with a postdevelopmental function. These mice have an increased bone density, which is somewhat unexpected for loss of a “bone inducer” molecule (Daluiski et al. 2001). The amino-terminal (1-34) region of PTH regulates osteoblast function, through its receptor PTHR1, in an ambiguous way. Permanent application stimulates only the early stages of osteoblast proliferation and suppresses the later stages. Intermittent PTH, in contrast, increases the numbers of mature, bone-forming osteoblasts (Karaplis and Goltzman 2000). PTH effects are therefore potentially anabolic and catabolic,
depending on the dose as well as on the changes of the dose over time. Sex steroids are crucial for the central regulation of bone remodeling. The interaction of estrogen, androgens, and their different receptors is so complex that it is beyond the scope of this article. In general, it can be said that estrogen and androgens can, in vitro, increase bone formation and decrease bone resorption by reciprocally influencing osteoblasts and osteoclasts via direct and indirect pathways (reviewed by Rickard et al. 1999). Leptin is a hormone that is produced by adipose tissue and regulates body weight via binding to hypothalamic receptors. Since it had long been recognized that a high BMI provides protection from osteoporosis, the group of G. Karsenty investigated whether mice deficient for leptin or the leptin receptor would have abnormal bone density. Both mutants had a two- to threefold increased bone density, due to an increased bone formation rate. No direct action of leptin on bone cells could be detected; instead, intracerebral administration of leptin strongly reduced bone density. The signaling from the hypothalamus to the osteoblast was shown to occur via the sympathetic nervous system and involved β2-adrenergic receptors (Takeda et al. 2002).

**Conditions with Abnormal Mineralization**

Abnormal mineralization may present either as under-mineralization (rachitic changes, osteomalacia) or as over-mineralization (increased density on radiographs, osteosclerosis). Hypophosphatasia (MIM 146300, MIM 171760, and MIM 241500) is a characteristic example of decreased mineralization. Three different types are distinguished on clinical grounds. The perinatally lethal form is characterized by (i) lack of ossification of calvarial bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios. The severe forms are transmitted as autosomal recessive to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.

Hyperostosis of calvarial and long bones; (ii) short, deformed extremities with absence of ossification of whole bones; (iii) respiratory distress due to short undermineralized ribs; and (iv) polyhydramnios.
The decisive common feature of all phenotypes, however, is an increased thickness of long-bone cortices with high mechanical stability. All mutations are situated in the first extracellular β-propeller module of the LRP5 protein. Several hypercholesterinemia-causing mutations have been found in the corresponding portion of the LDL receptor, proving its functional importance for this protein family. The effect of the first identified HBM mutation (G171V) is an increase in Wnt signaling, due to a diminished affinity of LRP5 to the antagonistic protein dkk, which results in higher osteoblast activity (Boyden et al. 2002). The investigation of sclerosteosis (MIM 269500) and van Buchem disease (MIM 239100) promises to shed light onto the obscure role of BMPs in bone homeostasis. Both diseases are characterized by an immense bone overgrowth, which leads to osteosclerosis and tall stature of varying degree. Loss-of-function mutations have been identified in the SOST gene in sclerosteosis. In the milder van Buchem disease, only a 52-kb deletion downstream of the SOST gene was detected, which might influence gene expression. SOST is a homolog of Gremlin, a BMP antagonist, and was shown to block bone formation in vitro (Janssens and Van Hul 2002).

Estrogen loss is the main cause of osteoporosis, a major health problem in industrialized countries. Accelerated bone loss during the postmenopausal phase is characterized by reduced osteoblast function and increased resorption by osteoclasts, whereas the slow decay typical of osteoporosis at higher ages is mainly due to impaired osteoblast activity. Mutations in estrogen receptor β and in the enzyme aromatase, which converts androgens to estrogen, lead to osteoporotic phenotypes (Smith et al. 1994; Bilezikian et al. 1998). Affected individuals are usually of tall stature, because of a delay of bone maturation and growth plate closure, which illustrates the importance of sex hormones for skeletal development.

Cross Talk between Osteoblast and Osteoclast

The model of a balanced competition between osteoblasts and osteoclasts in homeostasis predicts that a weakening of one cell type results in either bone accumulation or bone loss. This is demonstrated by a simple experiment: A specific ablation of mature osteoblasts by transgenic expression of herpes virus thymidine kinase under control of the osteocalcin promoter causes a ganciclovir-inducible and reversible bone loss, since bone resorption apparently proceeds normally (Corral et al. 1998). Although osteoblasts and osteoclasts are antagonists, they communicate with each other intensively. This is exemplified by the osteopetrotic (op) mouse mutant, which lacks macrophage-colony stimulating factor (M-CSF), a soluble growth factor that is produced by bone stromal cells and promotes proliferation of early osteoclast precursors (Felix et al. 1990). Yet M-CSF alone is not sufficient for osteoclast proliferation and differentiation. RANKL serves as a ligand for the receptor activator of NF-κB (RANK), a receptor that resides at the cell surface of osteoclast precursors (fig. 5). RANK initiates osteoclast differentiation via the NF-κB pathway, involving direct interaction with TRAF6, which is interconnected with other signaling pathways. Stromal cells, however, are also able to secrete osteoprotegerin (OPG), which is a truncated soluble RANK protein that can bind to RANKL and make it thus inaccessible to the osteoclast receptor. Whereas RANK knockouts are severely osteoprototic, OPG knockouts are osteoporotic, which highlights the crucial role of this signaling system for bone remodeling (Simonet et al. 1997; Kong et al. 1999). Most hormonal signals are not directly received by the osteoclast but are translated by the osteoblast via M-CSF and RANKL/OPG. An important exception is calcitonin, which acts directly upon osteoclasts to stop resorption (Baron et al. 1990). The other two hormones that regulate calcium homeostasis, PTH and 1,25-dihydroxyvitamin D₃, stimulate RANKL expression by osteoblasts (Quinn et al. 2001; Thomas et al. 2001). Interleukin-1 and tumor necrosis factor (TNF), in contrast, increase M-CSF secretion. This effect is antagonized by estrogen, which has additionally been shown to increase OPG production by osteoblasts (Hofbauer et al. 1999).

What about feedback from the osteoclast? There is evidence for production of Tgfβ by osteoclasts in response to estrogen and androgens (Robinson et al. 1996; Pederson et al. 1999). Furthermore, Tgfβ is bound to the bone matrix and, in a mechanism similar to the release of VEGF from the calcified cartilage, osteoclasts release it by bone resorption. Tgfβ stimulates OPG production by osteoblasts and osteoblast proliferation, so that a regulatory negative feedback loop is established (Takai et al. 1998; Dallas et al. 2002).

Disorders Caused by Disturbed Osteoblast-Osteoclast Cross Talk

Paget disease of bone (PDB) is a prominent example of signaling from the osteoclast to the osteoblast. In this condition, increased bone formation, which leads to cortical thickening, is driven by hyperactive osteoclasts. The dysregulation can be mimicked by viral infection of osteoclasts, and intracellular viruslike inclusions are frequently found in osteoclasts in PDB bone biopsies (Reddy et al. 2001). Excessive bone formation is prevented by administration of bisphosphonates that are known to selectively kill osteoclasts. Familial clustering of PDB suggests a strong genetic component. Indeed, different chromosomal loci could be linked to the disease. Evaluation of the candidate gene SQSTM1 on
Figure 5  Function and regulation of the osteoclast. Osteoblasts/stromal cells (yellow) regulate proliferation, differentiation, and activity of the osteoclast via RANKL and M-CSF. M-CSF binds to its receptor, c-fms, on mononuclear osteoclast precursors and stimulates their proliferation. RANKL stimulates differentiation into multinuclear resorbing osteoclasts via RANK, which transduces the signal with the help of TRAF6 and NFκB. As soon as the mature osteoclast attaches to the bone surface, it forms a ruffled border membrane containing large amounts of H⁺-ATPase complexes (green) and the chloride channel ClC-7 (red), which together transport HCl and acidify the resorption lacuna. Protons are provided by the cytosolic carbonic anhydrase type II (CAII). Acidic proteases like cathepsin k (blue circles) become active and degrade the bone matrix proteins (mainly collagen) and release bound growth factors, above all Tgfβ. Tgfβ is able to signal back to the osteoblast establishing a negative feedback loop by stimulating OPG expression. Degradation products are transported out of the cell by transcytosis. K = cathepsin k.

chromosome 5q31 revealed several mutations in familial and sporadic cases of PDB (Hocking et al. 2002; Laurin et al. 2002). This gene encodes p62 (sequestosome1), which has been described as a downstream effector of the RANK/TRA6 pathway (Sanz et al. 2000). It was furthermore shown that p62 binds ubiquitin and may be involved in protein degradation, which could explain the inclusion bodies seen in pagetic osteoclasts (Geetha and Wooten 2002).

A similar hereditary disorder with earlier onset is recessive juvenile Paget disease (MIM 239000). It was recently shown either to be caused by a deletion of the whole OPG-encoding TNFRSF11B gene or by a deletion of one amino acid in OPG, which prevents it from binding to RANKL (Cundy et al. 2002; Whyte et al. 2002). The dominant disorder familial expansile osteolysis (MIM 174810) is caused by a mutation in the RANK signaling peptide, which leads to intracellular accumulation and self-stimulation of the receptor (Hughes et al. 2000). Hence, all genes involved in the pathogenesis of
PDB and related disorders initially increase osteoclast activity by a dysregulation of the OPG/RANK/RANKL system and only secondarily lead to osteoblast stimulation. This is reflected by the finding that the disorders begin with a phase of increased resorption, which then entails osteoblast stimulation. As a consequence, extremely high levels of bone resorption are paralleled by high levels of bone formation at later stages.

Mutations in TGFβ1, which lead to Camurati-Engelmann syndrome (MIM 131300), prove the relevance of the Tgfβ feedback mechanism for bone homeostasis (Janssens et al. 2000; Kinoshita et al. 2000). Bone pain and walking problems are typical signs of this disorder. X-rays reveal a fusiform shape and progressive sclerosis of the long bone diaphyses, due to increased endosteal and periosteal bone formation. Most mutations are clustered around the disulfide bridge between the latency-associated peptides. The likely pathomechanism is premature activation of Tgfβ.

Another way to disturb the balance between osteoblasts and osteoclasts is exemplified by a rare recessive form of idiopathic osteolysis (MIM 605156). Patients with this disease have short stature, carpal and tarsal osteolyses, and an arthropathy, which eventually develops into ankylosis. The underlying genetic cause is inactivating mutations in matrix-metalloproteinase-2 (MMP-2) (Martignetti et al. 2001). This enzyme is exceptionally capable of digesting type IV collagen and is thought to regulate the inflammatory response. In bone, MMP-2 is produced by the osteoclast, but its function is elusive. A regulatory role has been discussed—for example, helping to release Tgfβ bound to the matrix or activating other proteases (Dallas et al. 2002). An MMP-2–deficient mouse model lacks a significant skeletal pathology and thus corresponds only weakly to the human phenotype (Narisawa et al. 1997).

**Osteoclast Differentiation and Function**

The differentiation of the mature, multinucleated osteoclast from its mononuclear hematopoietic precursor occurs in several well-defined steps. First, the formation of the precursor cells is dependent on PU.1, a hematopoietic transcription factor whose disruption in mice leads to a loss of lymph nodes, macrophages, and osteoclasts (Tondravi et al. 1997). Precursor proliferation is subsequently stimulated by M-CSF, which is produced by osteoblasts. The fate of these cells critically depends on Rank-signaling and on the c-Fos transcription factor (Wang et al. 1992). If either of them is missing, only macrophages and no osteoclasts develop from the mononuclear precursors, resulting in osteopetrotic phenotypes in mice. The osteoclasts-to-be then fuse and attach to the bone surface with the help of the vitronectin receptor, which triggers a signaling cascade involving Pyk2, c-Src, and c-Cbl to induce the conversion to a polarized resorbing osteoclast. This results in a massive fusion of acidic vesicles, with the osteoclast plasma membrane opposing the bone surface. The consequent membrane accumulation creates the so-called “ruffled membrane,” which contains high amounts of v-type H+ ATPases that pump protons into the tightly sealed resorption lacunae between osteoclast and bone surface. Strong extracellular acidification is crucial for bone resorption, since low pH is needed (i) to dissolve the hydroxyapatite of the bone tissue and (ii) to degrade the organic components of the bone matrix—above all, type I collagen—with acidic proteases like cathepsin k (fig. 5). After cathepsin k has cleaved the collagen fibrils, other proteases come into play that work under less acidic conditions. The degradation products are subsequently transferred to the basolateral pole of the cell by a transcytosis mechanism (reviewed by Väänänen et al. 2000).

**Disorders Caused by Osteoclast Dysfunction**

Changes in bone mass can be caused by reduced bone resorption due to a malfunction or a reduced number of osteoclasts. An extreme form of bone accumulation is seen in osteopetrosis. The majority of human osteopetrosis cases show high numbers of nonfunctional osteoclasts. The disease can be phenotypically subdivided into three (often overlapping) forms, all of which are based on defects in the osteoclast acidification machinery. Infantile malignant osteopetrosis usually begins shortly after birth and shows autosomal recessive inheritance (ARO [MIM 259700]). Characteristic signs are dense bones without a regular marrow cavity, bone-within-bone appearance, an increased fracture rate, hepatosplenomegaly, and cranial-nerve compression, which can lead to blindness and deafness (Gerritsen et al. 1994). The disorder is usually lethal if not treated early by bone-marrow transplantation. Mutations in TCIRG1, which encodes the a3 subunit of the osteoclast proton pump, are the major cause of recessive malignant osteopetrosis (Frattini et al. 2000; Kornak et al. 2000). Different types of mutations in different regions of the protein have been found in >50% of the cases, many leading to a truncation. The a subunit is the largest of the at least 13 subunits that form the v-type H+ ATPase and anchors the protein complex to the membrane. Only one a subunit is present per complex, so that the mutations can only have a loss-of-function effect. No clear genotype/phenotype correlation has so far been deduced.

An almost indistinguishable osteopetrotic phenotype is caused by mutations in CLCN7, which encodes the ubiquitously expressed CIC-7 chloride channel. It became clear that CIC-7 is functionally linked to the H+ ATPase, since it resides in the osteoclast ruffled membrane and transports chloride ions in parallel to the protons pumped into the resorption lacuna (Kornak et al. 2001). A truncating
Figure 6  Sandwich vertebra in ADOII. ADOII can be diagnosed by characteristic radiological findings. The radiograph shows the typical sandwichlike appearance of the vertebra by thickening of the endplates.

stop mutation and missense mutations have so far been identified in only a minority of cases. In contrast to TCIRG1 mutations, loss of functional CIC-7 in mice leads to a primary retinal degeneration that has also been described in human osteopetrosis cases.

Recently, a splice-site mutation in the Grey-lethal (GL) gene was found in a single ARO case (Chalhoub et al. 2003). The analysis of the corresponding mouse mutant indicated that gl, which harbors one putative transmembrane segment and is expressed in osteoclasts, osteoblasts, skin, and other tissues, might be involved in the arrangement of the osteoclast cytoskeleton.

A recessive form of intermediate severity is osteopetrosis with renal tubular acidosis (MIM 259730). It goes along with cerebral calcifications, which can result in mental retardation. The mutated protein is carbonic anhydrase type II, which is highly abundant in the osteoclast cytosol, synthesizing the protons transported by the H+ ATPase (Sly et al. 1983). High expression of this carbonic anhydrase in cerebral neurons and in renal intercalated cells explains the associated cerebral calcifications and renal dysfunction, respectively.

Autosomal dominant osteopetrosis type II (ADOII, also called “Albers-Schönberg disease” [MIM 166600]) is often not diagnosed before adulthood (fig. 6). The first signs are abnormal fractures or a suspicious appearance of the bones in routine x-rays, especially the so-called “sandwich vertebra” (Bénichou et al. 2000). Cranial-nerve compression is rare. Whereas in malignant recessive osteopetrosis bone resorption seems to be entirely abolished, bone accumulates in several steps during growth in ADOII to remain stable during later life. Recently, dominant missense mutations in the CLCN7 chloride channel gene were found, which are spread over the whole protein, often in the direct vicinity of recessive mutations (Cleiren et al. 2001). A mutation hotspot is the second cystathionine β-synthase domain, which is probably involved in protein-protein interaction.

The related sclerosing disorder, pycnodysostosis (MIM 265800), from which the painter Toulouse-Lautrec is thought to have suffered, is ascribed to mutations in cathepsin k, an acidic protease secreted by the osteoclast. The phenotype is milder than that of the recessive osteopetroses, with short stature, bone fragility, skull deformity, and hypoplasia of the clavicles being the main symptoms (Gelb et al. 1996). Cathepsin k is indispensable for bone resorption, since it is the only protease able to cleave the intact collagen triple helix, which renders it accessible for other osteoclast proteases—for example, MMP-9. Cathepsin k–deficient osteoclasts are therefore not able to degrade collagen but can still demineralize the underlying bone, showing that acid secretion is unaffected (Gowen et al. 1999).

Acknowledgment

We thank Marie-Christine de Vernejoul for kindly providing ADOII radiographs.

Electronic-Database Information

The URLs for data presented herein are as follows:


Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for postaxial polydactyly type I, preaxial polydactyly type IV, Greig cephalopolysyndactyly, dominant Pallister-Hall syndrome, TBS, TPTPS, EEC, limb-mammary syndrome, nonsyndromic SHFM, Hay-Wells syndrome, SHFM1, HOS, Okihiro syndrome, ulnar-mammary syndrome, acheiropodia, NPS, SPD, hand-foot-genital syndrome, amegakaryocytic thrombocytopenia and radioulnar synostosis, BDA1, BDB, BDC, chondrodysplasia [Grebe type], chondrodysplasia [Hunter-Thompson type], Du Pan syn-
drome, proximal symphalangism, multiple-synostosis syn-
drome, campomelic dysplasia, Ellis–van Creveld syndrome,
CCD, IWD, Langer mesomelic dysplasia, multiple cartilagi-
nous exostoses, tricho-rhino-phalangeal syndrome, Blom-
strand chondrodysplasia, Jansen type metaphyseal chondro-
dysplasia, enchondromatosis, achondroplasia, hypochondro-
dysplasia, thanatophoric dysplasia, autosomal dominant hypo-
phosphatemic rickets, recessive Robinow syndrome, achon-
drogenesis type II, hypochondrogenesis, SEDC, Kniest
dysplasia, Stickler dysplasia, otopspondyloepiphyseal dysplasia,
EDM2, EDM3, EDM1, pseudoachondroplasia, EDM5, EDM4,
diastrophic dysplasia, atelosteogenesis type 2, achondrogenesis type 1B, dyssegmental dysplasia [Silver-
man-Handmaker type], Schwartz-Jampel syndrome, hypo-
phosphatasia, CMD, OPS, HBM, ADOI, sclerosteosis, van
Buchem disease, juvenile Paget disease, familial expansile os-
teolysis, Camurati-Engelmann syndrome, idiopathic oste-
lysis, ARO, osteopetrosis with renal tubular acidosis, ADOII,
and pycnodysostosis)

Références

ADHR Consortium, The (2000) Autosomal dominant hypo-
phosphataemic rickets is associated with mutations in
FGF23. Nat Genet 26:345–348
Afzal AR, Rajab A, Fenske CD, Oldridge M, Elanko N, Ternes-
Pereira E, Tuysuz B, Murday VA, Patton MA, Wilkie AO,
Jeffery S (2000) Recessive Robinow syndrome, allelic to
dominant brachydactyly type B, is caused by mutation of
Akiyama H, Chaboissier MC, Martin JF, Schell A, de Crom-
brugge B (2002) The transcription factor Sox9 has essential
roles in successive steps of the chondrocyte differentiation
pathway and is required for expression of Sox5 and Sox6.
Genes Dev 16:2813–2828
Albrecht AN, Schwabe GC, Stricker S, Boddrich A, Wanker
EE, Mundlos S (2002) The synpolydactyly homolog (spdh)
mutation in the mouse—a defect in patterning and growth of
Arikawa-Hirasawa E, Watanabe H, Takami H, Hassell JR,
Yamada Y (1999) Perlecan is essential for cartilage and ce-
Arikawa-Hirasawa E, Wilcox WR, Le AH, Silverman N, Gov-
indraj P, Hassell JR, Yamada Y (2001) Dyssegmental dys-
plasia, Silverman-Handmaker type, is caused by functional
Bamshad M, Lin RC, Law DJ, Watkins WC, Krakowiak PA,
Moore ME, Franceschini P, Lala R, Holmes LB, Gebuhr TC,
Bruneau BG, Schinzel A, Seidman JG, Seidman CE, Jorde
LB (1997) Mutations in human TBX3 alter limb, apocrine
and genital development in ulnar-mammary syndrome. Nat
Genet 16:311–315
Selective internalization of the apical plasma membrane and
rapid redistribution of lysosomal enzymes and mannose 6-
phosphate receptors during osteoclast inactivation by cal-
Basson CT, Bachinsky DR, Lin RC, Levi T, Elkins JA, Souls
J, Grayzel D, Kroumposouz E, Traill TA, Leblanc-Straceski
J, Renault B, Kucherlapati R, Seidman JG, Seidman CE
and cardiac malformation in Holt-Oram syndrome. Nat Gen-
et 15:30–35
Basson CT, Huang T, Lin RC, Bachinsky DR, Weremowicz S,
Vaglio A, Bruzzone R, Quadrelli R, Lerone M, Romeo G,
Silengo M, Pereira A, Krieger J, Mesquita SF, Kamisago M,
Morton CC, Pierpont ME, Muller CW, Seidman JG, Seid-
man CE (1999) Different TBX5 interactions in heart and
limb defined by Holt-Oram syndrome mutations. Proc Natl
Acad Sci USA 96:2919–2924
Belin V, Cusin V, Viot G, Girlich D, Toutain A, Moncla A,
Vekemans M, Le Merrer M, Munnich A, Cormier-Daire V
(1998) SHOX mutations in dyschondrosteosis (Leri-Weil
syndrome). Nat Genet 19:67–69
Bell J (1951) On brachydactyly and symphalangism. In: Pen-
rose LS (ed) The treasury of human inheritance. Vol 5. Ox-
ford University Press, Cambridge, pp 1–31
Bellaiche Y, The I, Perrimon N (1998) Tout-velu is a Dro-
sophila homologue of the putative tumour suppressor EXT-
1 and is needed for Hh diffusion. Nature 394:85–88
Bellus GA, Gaudenz K, Zackai EH, Clarke LA, Szabo J, Fran-
comano CA, Muenke M (1996) Identical mutations in three
different fibroblast growth factor receptor genes in auto-
somal dominant craniosynostosis syndromes. Nat Genet 14:
174–176
Bellus GA, McIntosh I, Smith EA, Ayvsworth AS, Kaitila I,
Horton WA, Greenhaw GA, Hecht JT, Francomano CA
(1995) A recurrent mutation in the tyrosine kinase domain
of fibroblast growth factor receptor 3 causes hypochondro-
Bénichou OD, Laredo JD, de Vernejoul MC (2000) Type II
autosomal dominant osteopetrosis (Albers-Schönberg dis-
ease): clinical and radiological manifestations in 42 patients.
Bone 26:87–93
Bi W, Deng J, Zhang Z, Behringer RR, de Crombrugge B
22:85–89
Increased bone mass as a result of estrogen therapy in a man
Bowe AE, Finnegar R, Jan de Beur SM, Cho J, Levine MA,
phosphate transport and is a PHEX substrate. Biochem Bio-
phys Res Commun 284:977–981
Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick
due to a mutation in LDL-receptor-related protein 5. N Engl
J Med 346:1513–1521
Briggs MD, Chapman KL (2002) Pseudoachondroplasia and
euplephalysial dysplasia: mutation review, molecular
review, molecular interactions, and genotype to phenotype correlations. Hum
Mutat 19:465–478
Bruneau S, Johnson KR, Yamamoto M, Kuroiwa A, Duboule
D (2001) The mouse Hoxd13 gene, a polyanalyne
expansion similar to human type II synpolydactyly (SPD),
disrupts the function but not the expression of other Hoxd
genes. Dev Biol 237:345–353
mutations and human developmental syndromes. Am J Med
Genet 112:284–290


