MOLECULAR PATHOLOGY OF SKELETAL DEVELOPMENT

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Skeletal dysplasias are congenital disorders of morphogenesis, development and growth of the skeleton. Their clinical manifestations include short stature, abnormal body proportions and/or limb shape, deformity, and various complications in different organs. The disabilities determined by skeletal dysplasias range from short stature ("dwarfism") to severe limitations in daily activities, and the burden of disease ranges from chronic joint pain and neurologic complications to psychosocial stigmatization because of unusual appearance and deformity. The burden of disease imposed by skeletal dysplasias on affected individuals is high (Rimoin et al. 2002).

Skeletal dysplasias are genetically determined. Although most dysplasias are individually rare, there is great heterogeneity, and over 200 different phenotypes of dysplasias and dysostoses have been distinguished by a combination of clinical features and skeletal appearance on radiography (Rimoin 1998; Hall 2002). The cumulative incidence of skeletal dysplasias has been estimated as at least 1 in 5000 newborns (Connor et al. 1985; Orioli et al. 1986; Andersen 1989; Stoll et al. 1989; Cadle et al. 1996).

The establishment of a precise diagnosis is important for numerous reasons: prediction of adult height, accurate recurrence risk, prenatal diagnosis in future pregnancies and most importantly, clinical management including prevention of complications and disabilities. The diagnosis of a skeletal dysplasia has long been made solely on clinical and radiographic features. Because of the large number of different conditions, and the range of individual variability that each condition may present, a clinical-radiographic diagnosis is often just an approximation with a more or less large degree of uncertainty. It is important to identify the biochemical and genetic basis of an individual

skeletal dysplasia as a more safe ground for diagnosis and as a first step towards biology-based management and treatment (Unger 2002). Skeletal dysplasia research has initially been conducted by dedicated pediatricians and geneticists with an interest in biology. In more recent years, basic research groups have been attracted by the wealth of information to be gained. As a result of this joint effort, the study of the biological basis of skeletal dysplasias has provided information that is crucial for the diagnosis and management of affected individuals, and at the same time has illustrated the complex process of skeletal development with completely new insights. Skeletal dysplasia research has bridged a gap between the care for patients with rare diseases and basic biology.

Sulfate metabolism and skeletal development

The following section shall give a brief review on the discovery of the molecular basis of two distinct diseases, Achondrogenesis 1B (ACG1B) and Diastrophic Dysplasia (DTD), which were found to share a common pathogenetic mechanism. It is noteworthy that two approaches, the older pathogenetic and biochemical approach, and the newer genetic approach of positional cloning, reached similar conclusions at approximately the same time and ultimately converged.

Achondrogenesis is one of the most severe skeletal disorders in humans, and invariably lethal before or shortly after birth (Fig. 1, left). Marco Fraccaro coined the name *acondrogenesi* in 1952 for the condition he observed in a stillborn female with severe micromelia and marked histologic abnormalities of cartilage (Fraccaro 1952). The condition described shortly thereafter and also designated achondrogenesis by Grebe (Grebe 1952), is different although superficially similar to Fraccaro's achondrogenesis because of limb shortening, and has since become known as Grebe chondrodysplasia or Grebe syndrome (MIM 200700). Exact figures of the incidence of achondrogenesis are not available, but it is not exceedingly rare: reviews with large patient numbers have been published (Wiedemann et al. 1974; Schulte et al. 1978). In the 1970s, the heterogeneity of

achondrogenesis was recognized. Through a combination of radiological and histologic criteria, achondrogenesis type 1 (ACG1) (then also called Fraccaro-Houston-Harris type or Parenti-Fraccaro type) and type II (ACG2) (called Langer-Saldino type) were distinguished (Spranger et al. 1974; Yang et al. 1974). In 1976, it was found that chondrocytes of some ACG1 patients contained cytoplasmic inclusions (Yang et al. 1976). In the late 1980s, ACG 2 was found to be the consequence of structural mutations in collagen II and thus represented the severe end of the spectrum of the collagen II chondrodysplasias (Eyre et al. 1986; Godfrey and Hollister 1988; Godfrey et al. 1988; Spranger et al. 1994). Borochowitz and colleagues (Borochowitz et al. 1988) and van der Harten and colleagues (van der Harten et al. 1988) provided histologic criteria for the subdivision of ACG1 into 1A (with apparently normal cartilage matrix but inclusions in chondrocytes) and 1B (with abnormal cartilage matrix). Using these criteria, some cases from the earlier literature can be unequivocally diagnosed as type 1B, others as type 1A (Superti-Furga 1996).

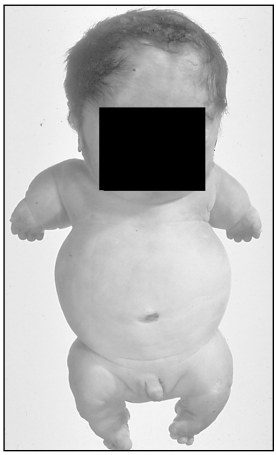

Fig. 1. Left: Achondrogenesis 1B. The limbs are severely shortened and the thorax is narrow, leading to respiratory insufficiency right after birth. Right: Diastrophic Dysplasia. The limbs are shortened, and in addition there are joint contractures with bilateral clubfeet.

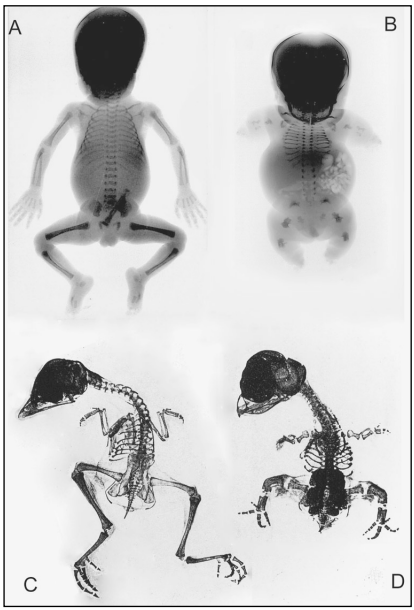

Fig.2. A: Radiography of a baby at gestational age 30 showing normal skeletal development. B: Radiography of the patient with achondrogenesis type 1B showing severe hypo-dysplasia of all skeletal elements, particularly of the long bones; the skull is relatively spared. C: alizarin-stained preparation of a chicken embryo with normal skeletal development. D: an embryo affected by the nanomelia mutation showing marked shortening of long bones. The pattern is reminiscent of that seen in achondrogenesis 1B. (C and D modified from Landauer, W.; J Hered 56:131-138, 1965)

In 1993, it was found that deficiency in aggrecan is the cause of nanomelia, a severe skeletal dysplasia in chicken (Li et al. 1993). Aggrecan is a large, highly sulfated proteoglycan very abundant in cartilage tissue. The skeletal morphology of aggrecan deficiency in chicken embryos was reminiscent of that of human achondrogenesis 1B (Fig. 2). Years earlier, B. Steinmann had examined cartilage collagens in ACG1B and found no abnormality. Thus, experiments were started to test the hypothesis that the basic defect in ACG1B was a deficiency in aggrecan. Indeed, cartilage extracts derived from a newborn with ACG1B were found to stain poorly with toluidine blue after gel electrophoresis, indicating a deficiency in sulfated proteoglycans, and cultured fibroblasts synthesized proteoglycans that could be marked with radiolabeled glycine or methionine but not with sulfate (Superti-Furga 1994b; Superti-Furga 1994a). When pulsed with 35SO4Z, fibroblasts of the same patient failed to synthesize phosphoadenosine-phosphosulfate (PAPS; Fig. 3, right part), the high-energy form of sulfate that is the indispensable substrate of biological sulfation.

These unusual findings did not confirm the hypothesis of a specific deficiency in aggrecan, but instead suggested a generalized defect in sulfate metabolism in achondrogenesis 1B that impaired the correct sulfation of cartilage proteoglycans (including aggrecan); as a consequence, cartilage tissue was weaker than normal, and growth was stunted. The first interpretation of these findings was that of a sulfate activation defect (Superti-Furga 1994b; Superti-Furga 1994a) analogous to that seen in the brachymorphic mouse. However, when achondrogenesis fibroblasts were homogenized rather than assayed intact, their ability to activate sulfate was normal. What caused the block in sulfate utilization in ACG1B? The possibility of a sulfate uptake defect was considered, but a single sulfate uptake assay failed to show differences between patient cells and control cells (unpublished); in retrospect, the conditions for that assay were not suitable to show the sulfate uptake defect that is indeed present.

Diastrophic Dysplasia (DTD; Fig. 1) was one of the first skeletal dysplasias to be clearly delineated on a phenotypic and radiographic ground. It was described by Lamy and Maroteaux in 1960 as "bone anomalies that simulate achondroplasia during the first years of life but show a quite different evolution"(Lamy and Maroteaux 1960): they reported 3 own observations and identified 11 other reports in the literature that appeared to describe the same condition. The name diastrophic dwarfism refers to the "twisted" body shape of affected individuals. DTD is a severe, though usually nonlethal, form of skeletal dysplasia with marked short stature (both the trunk and the limbs are shortened, but the limbs more severely so), bilateral clubfoot, cleft palate, characteristic hand deformities, changes of the ear pinnae, progressive kyphoscoliosis of the spine, and joint stiffness (Fig.1, right). Attempts at understanding the biochemical basis of this disorder in the 1970s and 1980s were unsuccessful. Thus, a genetic approach was chosen, making use of the fact that the disorder is particularly frequent in Finland. The locus responsible for this disorder was initially mapped to chromosome 5 (Hastbacka et al. 1990). A more precise mapping to 5q32-q33.1 was obtained using linkage disequilibrium with polymorphic markers on chromosome 5 in the Finnish population (Hastbacka et al. 1992). A candidate cDNA encoded by a gene from

that region (Hastbacka et al. 1994) was noted to have homology with sat-1, a rat hepatocyte sulfate transporter. The hypothesis that diastrophic dysplasia was associated with impaired sulfate transport was confirmed by the demonstration of reduced sulfate uptake in cultured fibroblasts from patients with DTD and by the demonstration of mutations in the candidate gene in such individuals (Hastbacka et al. 1994). The gene product was then called diastrophic dysplasia sulfate transporter (DTDST). Recognition that the gene responsible for DTD encoded a sulfate transporter was a surprise because there was no prior experimental evidence of abnormal sulfate or proteoglycan metabolism in that disorder.

When the sulfate transporter responsible for diastrophic dysplasia was cloned, we decided to investigate on the relationship between ACG1B and DTD. Indeed, fibroblast cocultivation studies, sulfate uptake studies, and, finally, DTDST mutation analysis showed that the primary defect in ACG1B was a sulfate uptake defect caused by DTDST mutations and thus, ACG1B and DTD were allelic disorders (Superti-Furga et al. 1996). The biochemical and genetic tests showed that the two dysplasias were essentially a severe and a milder form of the same disorder – biochemistry had met genetics.

Identification of a common basis for achondrogenesis 1B and diastrophic dysplasia brought sulfate metabolism in the spotlight of scientific interest. Two other disorders were found to be caused by mutations in the sulfate transporter: the severe dysplasia atelosteogenesis 2 (Hastbacka et al. 1996), and a mild disorder called multiple epiphyseal dysplasia and characterized by normal stature, mild joint contractures, and early degenerative joint disease (Superti-Furga et al. 1999; Superti-Furga et al. 2000; Ballhausen et al. 2002). Surprisingly, this mildest expression of DTDST defects appears to be quite common, as it is associated preferentially with the most frequent of DTDST mutations, R279W. A defect in PAPS synthase, leading to a block in the metabolic activation of sulfate, which takes place within the cell after sulfate has been taken up through DTDST (Fig. 3, right part), has been linked to yet another skeletal dysplasia, a variant of spondyloepi-metaphyseal dysplasia (ul Haque et al. 1998).

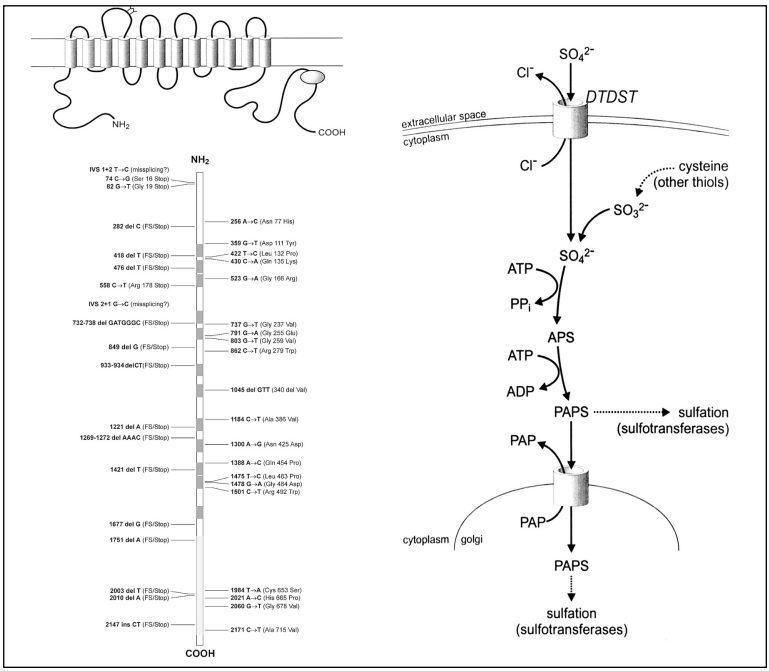

Fig. 3. Top left: schematic representation of the sulfate transporter. Bottom left: overview on mutations identified in individuals affected by achondrogenesis 1B, atelosteogenesis 2, diastrophic dysplasia and multiple epiphyseal dysplasia. Right: schematic representation of the steps of sulfate metabolism within the cell.

The structure of the sulfate transporter DTDST, with twelve transmembrane domains and a C-terminal hydrophobic region (Fig.3., top left), is found in two other molecules that have been identified as ion transporters: one is PDS, a chloride-iodide antiporter involved in Pendred Syndrome, a genetic form of thyroid disease associated with deafness (Everett et al. 1997; Scott et al. 1999); the second is CLD, a chloride transporter involved in yet another genetic disorder, Congenital Chloride Diarrhea (Hoglund et al. 1996; Everett and Green 1999; Haila et al. 2000). It is intriguing that each of the three related molecules causes a distinct genetic disorder in different organs – the thyroid, the intestine, and the skeleton.

An important practical consequence of this research has been the development of a reliable molecular diagnostic test. Our laboratory has been able to confirm the diagnosis by mutation analysis in well over hundred individuals worldwide, including children and adults, but also in samples from pregnancies ended prematurely because of severely affected fetuses. Molecular diagnostic confirmation has allowed giving adequate prognostic information and genetic counseling (Fig.3, bottom left; Rossi and Superti-Furga 2001). Couples who have had a child affected by achondrogenesis 1B or diastrophic dysplasia have a 1/4 probability of having another affected child at each pregnancy. In spite of the higher probability that the child will not be affected (3/4), this notion may prevent the couple from having further children. In this context, the prospect of reliable first-trimester prenatal diagnosis is highly valued, and many families have taken advantage of the prenatal testing possibilities to fulfill their family planning (Rossi and Superti-Furga 2001).

Although diagnosis and primary prevention are significant goals that have been achieved so far, pharmacological treatment remains a long-term goal. Again, a serendipitous finding pointed one possible way (Superti-Furga 2001). At one point, we wanted to study whether the sulfate transporter DTDST played a role in the renal tubular handling of sulfate. Did patients with diastrophic dysplasia have lower plasma sulfate, perhaps as a consequence of renal loss of sulfate? The answer to this question is no: sulfate in plasma and urine is normal in individuals with diastrophic dysplasia. However, during this study we observed that some patients with generalized tubular dysfunction (the renal Fanconi syndrome) had markedly increased sulfate in plasma. Apparently, this was not the consequence of the renal disease, but simply a side effect of a drug they were taking – cysteamine – for their primary disorder, cystinosis. The oxidation of this sulfur-containing drug resulted in increased production of sulfate in the body. We speculated that oxidation of cysteamine to sulfate would occur within cells, and there were data in the literature showing that in cell culture and under special circumstances, sulfate present on proteoglycans could be derived from thiols such as cysteine. Could we bypass the DTDST step using thiols to stimulate sulfate production within the cell

(Fig.3, right part)? Using cell cultures from affected individuals, Antonio Rossi found that the addition of sulfur-containing compounds such as cysteine, cysteamine or N-acetylcysteine can indeed replenish the intracellular sulfate pool and increase sulfation of newly synthesized proteoglycans (Rossi et al. 1997; Rossi et al. 1998; Rossi et al. 2001). These observations suggested that thiols have a therapeutic potential in DTDST disorders.

In order to understand the pathogenesis of DTDST disorders more precisely and to explore the therapeutic potentials of sulfur-containing compounds, A. Rossi has undertaken the challenge of engineering a mouse model that would reproduce the human disease accurately enough to allow for therapeutic experiments. A DTDST mutation that we had observed to produce marked, but non-lethal disease in children has been genetically inserted into laboratory mice with a complex procedure of genetic engineering. In the summer of 2002, the work has been successful, as mice homozygous for the mutation where obtained that show features typical of a skeletal dysplasia, including reduced size and restricted joint movements. In the next years, the "DTD mouse" should tell us whether medical treatment could be used to prevent joint degeneration and pain and improve skeletal growth.

Skeletal dysplasias as a paradigm of developmental biology: development of a molecular-pathogenetic classification

The Achondrogenesis – Diastrophic Dysplasia family is but one of many exciting research areas in the field of skeletal dysplasias. The accumulation of knowledge on genes and proteins responsible for genetic disorders of the skeleton has turned the skeletal system into a unique biologic model. Until recently, approach to the complexity of skeletal dysplasias has been at the clinical or radiographic levels only. We speculated that the multitude and variety of genes and proteins would allow for a molecular and pathogenetic classification that would bridge between the pediatric clinic and the biology laboratory. Such a classification might assist in identifying metabolic pathways active in cartilage and bone, and their regulatory mechanisms; in identifying

cellular signaling networks and gene expression sequences implicated in skeletal development; in identifying other elements in those networks and sequences as candidate genes for genetic disorders; and in facilitating the integration of data coming from spontaneous and genetically engineered mouse mutants. More generally, a molecular classification might provide a knowledge framework accessible to physicians as well as to basic scientists and thus to facilitate communication between clinical genetics and pediatrics and the basic sciences, and ultimately help in developing diagnostic strategies and stimulate the design and exploration of new therapeutic possibilities.

As a basis for the development of the new classification, we adopted the functional classification of proteins in *S. cerevisiae, C. elegans* and disease-related human genes and proteins proposed in the 8th edition of The Metabolic and Molecular Bases of Inherited Disease (Jimenez-Sanchez et al. 2001). This classification was adapted to allow for the peculiarities intrinsic to skeletal biology. We grouped molecular defects as follows (Superti-Furga et al. 2001):

Group 1: Defects in extracellular structural proteins
Group 2: Defects in metabolic pathways (including enzymes, ion channels, transporters)
Group 3: Defects in folding, processing and degradation of macromolecules
Group 4: Defects in hormones and signal transduction mechanisms
Group 5: Defects in nuclear proteins and transcription factors
Group 6: Defects in oncogenes and tumor suppressor genes
Group 7: Defects in RNA and DNA processing and metabolism

Skeletal disorders with a well documented genetic and biochemical basis have been assigned to one of these groups. The interpretation proposed in the original papers has been used as the basis for classification, and other literature pertinent to the proposed molecular mechanism(s) has been consulted when the original description of the genetic defect fell short of suggesting a plausible pathogenetic mechanism or when new data has been published subsequently.

Table 1. Classification of genetic disorders of the skeleton by molecular and pathogenetic criteria - The first column lists the responsible genes or proteins; the second column the inheritance pattern; and the third, the associated clinical phenotypes (modified from Superti-Furga, Bonafé and Rimoin, Am J Med Genet 106:282-293, 2001).

Group 1: Defects in extracellular structural proteins		
COL1A1, COL1A2 (Collagen 1 α1, α2 chain)	AD	Family: Osteogenesis im perfecta
COL2A1 (Collagen 2 α1 chain)	AD	Family: achondrogenesis 2, hypochondrogenesis, congeni tal spondyloepiphyseal dyspla sia (SEDC), Kniest, Stickler arthro-ophtalmopathy, familial osteoarthritis, other variants
COL9A1, COL9A2, COL9A3 (Collagen 9 α1, α2, α3 chain)	AD	Multiple epiphyseal dysplasia (MED; two or more variants)
COL10A1 (Collagen 10 α1 chain)	AD	Metaphyseal Dysplasia Schmid
COL11A1, COL11A2 (Collagen 11 α1, α2 chain)	AR, AD	Oto-spondylo-megaepiphyseal dysplasia (OSMED); Stickler (variant), Marshall syndrome
COMP (cartilage oligomeric matrix protein)	AD	Pseudoachondroplasia, multiple epiphyseal dysplasia (MED, one form)
MATN3 (matrilin-3)	AD	Multiple epiphyseal dysplasia (MED; one variant)
Perlecan	AR	Schwartz-Jampel type 1; dyssegmental dysplasia

Group 2: Defects in metabolic pathways (including enzymes, ion channels, and transporters)		
TNSALP (tissue non-specific alkaline phosphatase)	AR, AD	Hypophosphatasia (several forms)
ANKH (pyrophosphate transporter)	AD	Craniometaphyseal dysplasia
DTDST /SLC26A2 (diastrophic dysplasia sulfate transporter)	AR	Family: achondrogenesis 1B, atelosteogenesis 2, diastrophic dysplasia, recessive multiple epiphyseal dysplasia (rMED)
PAPSS2, phosphoadenosine-phosphosulfate-synthase 2	AR	Spondylo-epi-metaphyseal dysplasia Pakistani type

TCIRG1, osteoblast proton pump subunit (acidification defect)	AR	Severe infantile osteopetrosis
ClC-7 (chloride channel 7)	AR	Severe osteopetrosis
Carboanhydrase II	AR	Osteopetrosis with intracranial calcifications and renal tubular acidosis
Vitamin K-epoxide reductase complex	AR	Chondrodysplasia punctata with vitamin K-dependent coagulation defects
MGP (matrix Gla protein)	AR	Keutel syndrome (pulmonary stenosis, brachytelephalangism, cartilage calcifications and short stature)
ARSE (arylsulfatase E)	XLR	X-linked chondrodysplasia punctata (CDPX1)
3-β-hydroxysteroid-dehydrogenase	XLD	CHILD syndrome
3-β-hydroxysteroidD(8)D(7)- isomerase	XLD	X-linked chondrodysplasia punctata, Conradi-Hünermann type (CDPX2); CHILD syndrome
PEX7 (peroxisomal receptor/ importer)	AR	Rhizomelic chondrodysplasia punctata 1
DHAPAT (Di-hydroxy-acetonphosphate-acyltransferase, peroxisomal enzyme)	AR	Rhizomelic chondrodysplasia punctata 2
Alkyl-Di-hydroxy-diacetonphosphate synthase (AGPS; peroxisomal enzyme)	AR	Rhizomelic chondrodysplasia punctata 3

Group 3: Defects in folding and degradation of macromolecules

Sedlin (endoplasmic reticulum protein with unknown function)	XR	X-linked spondyloepiphyseal dysplasia (SED-XL)
Cathepsin K (lysosomal proteinase)	AR	Pycnodysostosis
Lysosomal acid hydrolases and transporters (sulfatase, glycosidase, translocase, etc.)	AR,XLR	Lysosomal storage diseases: mucopolysaccharidoses, oligosaccharidoses, glycoproteinoses (several forms)
Targeting system of lysosomal enzymes (GlcNAc-1-phosphotransferase)	AR	Mucolipidosis II (I-cell disease), mucolipidosis III
MMP2 (matrix metalloproteinase 2) arthropathy and osteolysis syndrome)	AR	Torg type osteolysis (nodulosis

Group 4: Defects in hormones and signal transduction mechanisms

25-α-hydroxycholecalciferol-1-hydroxylase	AR	Vitamin D-dependent rickets type 1 (VDDR1)
1,25-α-dihydroxy-vitamin D3 receptor	AR	Vitamin D-resistant rickets with end-organ unresponsiveness to vitamin D3 (VDDR2)
CASR (calcium "sensor"/ receptor)	AD	Neonatal severe hyperparathyroidism with bone disease (if affected fetus in unaffected mother); familiar hypocalciuric hypercalcemia
PTH/PTHrP receptor	AD (activating mutations)	Metaphyseal dysplasia Jansen
	AR (inactivating mutation)	Lethal dysplasia Blomstrand
	AD or somatic mutations	Enchondromatosis
GNAS1 (stimulatory Gs alpha protein of adenylate cyclase)	AD	Pseudohypoparathyroidism (Albright Hereditary Osteodystrophy and several variants) with constitutional haploinsufficiency mutations; McCune-Albright syndrome with somatic mosaicism for activating mutations
PEX proteinase	XL	Hypophosphatemic rickets, X-linked semi-dominant type (impaired cleavage of FGF23)
FGF23, fibroblasts growth factor 23	AD	Hypophosphatemic rickets, autosomal dominant type (resistance to PEX cleavage)
FGFR1 (fibroblast growth factor receptor 1)	AD	Craniosynostosis syndromes (Pfeiffer, other variants)
FGFR2	AD	Craniosynostosis syndromes (Apert, Crouzon, Pfeiffer; several variants)
FGFR3	AD	Thanatophoric dysplasia, achondroplasia, hypochondroplasia, SADDAN; craniosynostosis syndromes (Crouzon with acanthosis nigricans, Muenke nonsyndromic craniosynostosis)

ROR-2 (“orphan receptor tyrosine kinase”)	AR	Robinow syndrome
	AD	Brachydactyly type B
TNFRSF11A (receptor activator of nuclear factor kB; RANK)	AD	Familial expansile osteolysis
Osteoprotegerin (TNFRSF11B; RANK receptor, soluble)	AR	Idiopathic Hyperphosphatasia with osteoectasia; Juvenile Paget disease
TGFb1	AD	Diaphyseal Dysplasia (Camurati-Engelmann)
CDMP1 (cartilage-derived morphogenetic protein 1)	AR	Acromesomelic dysplasia Grebe/Hunter-Thompson;
	AD	Brachydactyly type C
Noggin (“growth factor”, TGF antagonist)	AD	Multiple synostosis syndrome; synphalangism and hypoacusis syndrome
DLL3 (delta-like 3, intercellular signaling)	AR	Spondylo-costal dysostosis (one form)
IHH (Indian hedgehog signal molecule)	AD	Brachydactyly A1
C7orf2 (orphan receptor)	AR	Acheiropodia
SOST (sclerostin; cystine knot secreted protein)	AR	Sclerosteosis, van Buchem disease
LRP5 (LDL receptor-related protein 5)	AR	Osteoporosis-pseudoglioma syndrome
WISP3 (growth regulator/growth factor)	AR	Progressive pseudorheumatoid dysplasia

Group 5: Defects in nuclear proteins and transcription factors

SOX9 (HMG-type DNA binding protein/ transcription factor)	AD	Campomelic dysplasia
Gli3 (zinc finger gene)	AD	Greig cephalopolysyndactyly, polydactyly type A and others, Pallister-Hall syndrome
TRPS1 (zinc-finger gene) (types 1 to 3)	AD	Tricho-Rhino-Phalangeal syndrome
EVC (leucine-zipper gene	AR	Chondro-ectodermal dysplasia (Ellis-van Creveld)
TWIST (helix-loop-helix transcription factor)	AD	Craniosynostosis Saethre-Chotzen
P63 (p53 related transcription factor)	AD	EEC syndrome, Hay-Wells syndrome, limb-mammary syndrome, split hand – split foot malformation (some forms)

CBFA-1 (core binding factor A 1; runt-type transcription factor)	AD	Cleidocranial dysplasia
LXM1B (LIM homeodomain protein)	AD	Nail-patella syndrome
DLX3 (distal-less 3 homeobox gene)	AD	Tricho-dento-osseous syndrome
HOXD13 (homeobox gene)	AD	Synpolydactyly
MSX2 (homeobox gene)	AD (gain of function)	Craniosynostosis, Boston type
	AD (loss of function)	Parietal foramina
ALX4 (homeobox gene)	AD	Parietal foramina (cranium bifidum)
SHOX (short stature – homeobox gene)	X/Y, Pseudoautosomal	Léri-Weill dyschondrosteosis, i diopathic short stature?
TBX3 (T-box 3, transcription factor)	AD	Ulnar-mammary syndrome
TBX5 (T-box 5, transcription factor)	AD	Holt-Oram syndrome
EIF2AK3 (transcription initiation factor kinase)	AR	Wolcott-Rallison syndrome (neonatal diabetes mellitus and spondyloepiphyseal dysplasia)
NEMO (NFkB essential modulator; kinase activity)	XL	Osteopetrosis, lymphedema, ectodermal dysplasia and immunodeficiency (OLEDAID)

Group 6: Defects in oncogenes and tumor suppressor genes

EXT1, EXT2 (exostosin-1, exostosin-2; heparan-sulfate polymerases)	AD	Multiple exostoses syndrome types1, type 2
SH3BP2 (c-Abl-binding protein)	AD	Cherubism

Group 7: Defects in RNA and DNA processing and metabolism

RNAse MRP-RNA component	AR	Cartilage-Hair-Hypoplasia (icluding metaphyseal dysplasia without hypotrichosis)
ADA (adenosine deaminase)	AR	Severe Combined Immunodeficiency (SCID) with (facultative) metaphyseal changes
SMARCAL1 “chromatin regulator”	AR	Schimke immuno-osseous dysplasia

The traditional "Nomenclature of constitutional disorders of the skeleton" is based on phenotypic features only (radiography, clinical findings, and inheritance pattern) (Rimoin 1998; Hall 2002). Many pediatricians and geneticists consider it somewhat arbitrary and difficult to use. The molecular classification confirms that there is a great molecular complexity at the basis of what we observed phenotypically, and thus retrospectively confirms, at least in part, what had been classified empirically. On the other hand, the notion that multiple phenotypes may be caused by mutations in a single gene seems to be the rule rather than the exception. One of the consequences for future work will be that great caution should be used when suggesting the existence of "new" disorders, and biochemical and molecular evidence should be sought.

The individual disorders we have been able to classify are listed in Table 1. We were surprised by the wealth of data available, and by the consistency with which disorders could be classified. The most rewarding feature was the observation of emerging pathways; for example, there are two disorders related to sulfate metabolism (DTDST and PAPSS); three related to osteoclast acidification (CA-II, ClC7, and TCIRG1); and three related to pyrophosphate metabolism (ANKH/CMD, TNSALP, and nucleotide pyrophosphorylase). Group 7 consists of three disorders that share an immunologic and skeletal phenotype; all three disorders are routed in defects related to processing of DNA and RNA(Hirschhorn 1995; Ridanpaa et al. 2001; Boerkoel et al. 2002; Bonafe et al. 2002). An intriguing finding is the possible relationship between two genetic disorders that may simulate "adult-type" degeneration: the Osteoporosis-Pseudoglioma syndrome (Gong et al. 2001) and Progressive Pseudorheumatoid Dysplasia (Hurvitz et al. 1999). Both conditions involve a *wnt*-signalling pathway. Other pathways or regulatory cascades can be predicted, e.g., involving embryonic segmentation (Dll3, Notch, and other genes being candidates). The identification of such pathways or cascades points to "clue" processes and suggests that other steps in those pathways may also be relevant to disease states. A for pharmacologic interventions and thus become relevant for common degenerative diseases of the adult such as osteoporosis and arthritis.

If research into the molecular basis of a single disorder or group of disorders means going into the depth of a particular area and working out the details as fully as possible, drafting the classification could be compared to using a helicopter to obtain a general picture from greater height. Although it is not experimental work at the bench, but rather compilation at the writing desk (or laptop), it is no less interesting and challenging. Response from colleagues and fellows to the published version has been positive, some reporting that the classification had offered them a new framework and opened for them new perspectives. Inherent to every classification is simplification, as well as the limitation of knowledge available at a given time: biology is more complex and subtle, and undoubtedly, new results may change the current grouping significantly. The classification is only a tool; all tools get blunted and twisted with heavy use, and it is by their use that they become familiar and valued, until they get replaced by new ones.

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