CURRENT CONCEPTS REVIEW

GENES AND ORTHOPEDICS : FROM GENE TO CLINIC AND VICE VERSA

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Recent advances in molecular biology have greatly helped in understanding the mechanisms involved in normal skeletal morphogenesis. Multiple genes involved in normal skeletal development have been identified, but several others still await discovery. Mutations in these genes are often responsible for the congenital skeletal malformations that we see in the orthopedic clinics. In this overview we would like to emphasize the importance of the interaction between orthopaedic surgeons, molecular biologists and geneticists.

Keywords : skeletal development ; congenital orthopedic malformations.
Mots-clés : développement du squelette ; malformations orthopédiques.

INTRODUCTION

The embryonic development of the skeleton and limbs is an intriguing and complex process, which involves a cascade of molecular interactions. More specifically, differentiation, proliferation and programmed cell death play an important role. Much of what we know about the general mechanism underlying skeletal morphogenesis is the result of animal studies. The consequences of spontaneous or induced mutations in the fruitfly (*Drosophila melanogaster*), worm *(Caenorhabditis elegans*), zebra fish (*Danio rerio*), frog *(Xenopus laevis*), chicken and mouse have lead to the identification of a multitude of genes involved in normal skeletal development (39, 41, 44, 45). Congenital deformations in humans, isolated or as part of a syndrome, also provide us with the unique opportunity to identify novel genes. Recent technical advances in molecular biology and the completion of the Human Genome Project (38, 61), which involved sequencing the three million base pairs of the human genome, have significantly facilitated the possibility to locate and identify genes responsible for normal skeletal development. Further characterization of these genes and elucidation of the function of the corresponding proteins will undoubtedly provide us with more information regarding their role in normal limb- and skeletal development.

In this review, we present a brief overview of the current knowledge of the molecular aspects of skeletal disease and illustrate how the recent advances in genetics may have practical implications for patients with orthopaedic problems.

Etiology of congenital malformations

Over 6000 human disorders exhibit simple gene unifactorial or *Mendelian inheritance*. A disorder determined by a gene on an autosome is said to show *autosomal inheritance,* whereas a disorder determined by a gene on one of the sex chromo-

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somes is said to show *sex-linked inheritance*. A *dominant* trait manifests itself in a *heterozygote,* a person possessing both the abnormal or *mutant* allele and the normal allele. The clinical features in dominant traits can vary from person to person. This is known as *variable expressivity.* In some individuals of families with autosomal dominant traits, the presence of the mutation can go undetected ('skip a generation'), which is known as *reduced penetrance*. In autosomal dominant disorders an affected person usually has an affected parent. Sometimes a disorder can appear in an individual whose parents (and all the previous generations) are unaffected. This sudden appearance of a condition due to a mistake occurring in the transmission of a gene is called a *new mutation* (*de novo* mutation). *Recessive* disorders are only manifest when the mutant allele is present in a double dose (*homozygosity*). Both parents of the affected person are obligate heterozygous for the mutation. They are perfectly healthy *carriers*. Many disorders demonstrate a type of inheritance that is not similar to any recognised Mendelian pattern (for instance congenital dislocation of the hip). This pattern of inheritance is referred to as *multifactorial*. Both genes and environmental factors play a role in these disorders.

When searching for the possible causes of congenital malformations, four categories must be considered.

1. Sometimes, a clear exogenous insult can be identified as the main causative factor. Examples include the limb anomalies caused by thalidomide, congenital malformations caused by maternal illness (infections, hypertension, diabetes, toxemia) and transverse limb deficiencies caused by early chorion villus sampling with fetal vascular shock. Poland syndrome (unilateral symbrachydactyly with ipsilateral aplasia of the pectoralis major) is probably due to a primary defect in the development of the subclavian artery.
2. In some Mendelian inherited conditions, a single major gene can be identified, for example the *HOXD13* mutations in synpolydactyly (1, 47).
3. Chromosome studies in patients with a congenital malformation occasionally reveal a chromosomal aberration. These include abnormalities in chromosome number (aneuploidies, e.g. trisomy 13 and trisomy 21, which give rise to polydactyly and brachydactyly respectively) or abnormalities in chromosome structure (for example the ring chromosome 13 syndrome which is accompanied by absence of the thumb).
4. The great majority of congenital malformations however, are caused by a combination of genetic and environmental factors. In these cases patients have a genetic liability to develop a disease, but an environmental factor is also necessary.

We will further focus our attention on congenital disorders with an underlying genetic defect.

How can we find novel genes involved in skeletal malformations ?

Several approaches can be used in order to detect genes involved in human disease (11, 12). Sometimes information about the underlying biochemical defect is used. Using a *functional cloning* approach, the identification of a gene is based on pre-existing information about the underlying biochemical defect. No reference is made to any chromosomal map position or sequence information. *Positional cloning* assumes no functional information and must locate the responsible genes on the basis of map position. In a first step, linkage analysis of multiple affected families can assist in mapping the disease gene on a specific chromosome. The identification of patients with chromosomal aberrations (chromosomal deletions, inversions, translocations, duplications,...) is often of great help. After mapping the cytogenetic aberration to its correct location on the chromosome, the candidate interval is narrowed down until the gene is identified. The genetic tools used mainly consist of previously constructed chromosomal maps and sequence information. All these data can be found at various sites on the World Wide Web. The completion of the Human Genome Project greatly facilitates the search for genes involved in human disease. The *candidate gene* approach makes use of

information of already known genes. Features of the disease are compared with those of the gene and, depending on the results of this comparison, a specific gene is considered a good candidate or not. Mutation analysis of this gene in affected patients may prove that this gene is indeed responsible for the observed phenotype. The *positional candidate gene* approach combines the two latter methods. First the disease locus is mapped to the correct chromosomal region, followed by a search of this interval to see whether any functionally interesting candidate genes have been positioned there. In our search for genes involved in skeletal malformations, information derived from other species is often of great help. Several mouse models with distinct phenotypes have been described (39, 41). Sometimes a human limb deformity shows great resemblance with that of a previously described mouse. If the genetic defect in the mouse is known, identification of its human counterpart is possible (for example the identification of *hoxa13* mutations in the *hypodactyly* mouse and the subsequent identification of human *HOXA13* mutations in Hand-Foot-Genital-Syndrome (42, 43)). Figure 1 illustrates how currently all the available data and techniques can be combined in searching for human disease genes. There is a clear interplay between clinical work, computer analysis ("cyber cloning") and laboratory benchwork. It is obvious that the advances in the Human Genome Project greatly speed up this approach.

Molecular genetics of skeletal disease : an overview

A detailed description of all the disorders given below can be found in the OMIM database (Online Mendelian Inheritance in Man ; http://www.ncbi.nlm.nih.gov/Omim/). Table 1 gives an overview of some congenital malformations with orthopedic implications. The underlying genetic defect, if known, is indicated together with the mode of inheritance (AD = autosomal dominant ; AR= autosomal recessive) and the OMIM number. Neuromuscular and metabolic disorders were not included.

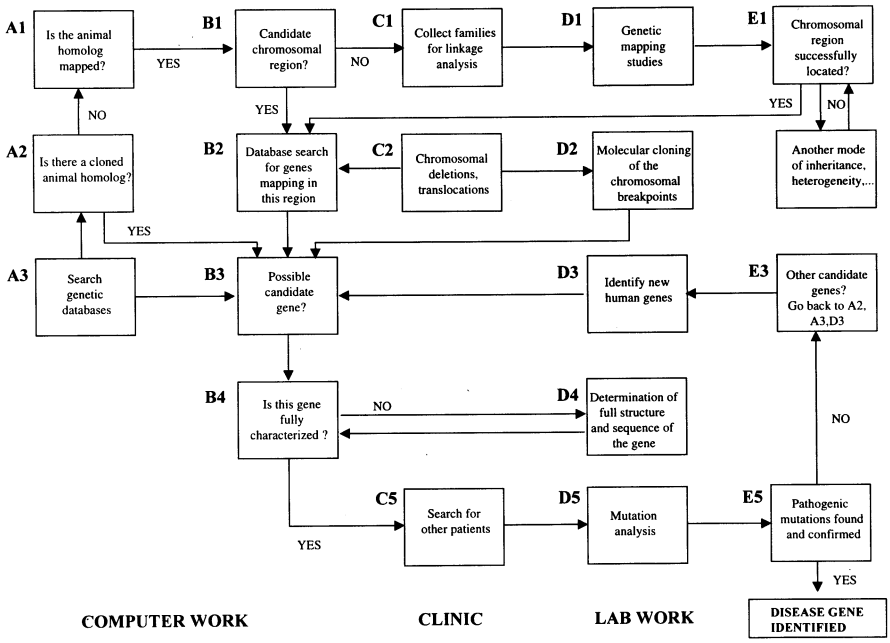

Fig. 1. — This flowchart illustrates the important interplay between clinical work, laboratory benchwork and computer analysis. The key step is to arrive at the point where you can identify a suitable candidate gene (Step B3). This candidate gene can subsequently be tested for mutations in other affected individuals. (Figure adapted with permission from Figure 15.1 in Tom Strachan & Andrew P. Read, Human Molecular Genetics 2, Second edition, 1999, BIOS Scientific Publishers, Ltd).

Table 1

Disease	Inheritance	Causative gene	OMIM
SKELETAL DYSPLASIAS			
Achondrogenesis type I	AR	*DTDST*	600972
Achondrogenesis type II	AR	*COL2A1*	200610
Achondroplasia	AD	*FGFR3*	100800
Campomelic dysplasia	AR	*SOX9*	114290
Cleidocranial dysostosis	AD	*RUNX2*	119600
Diastrophic dysplasia	AR	*DTDST*	222600
Grebe chondrodysplasia	AR	*CDMP1*	200700
Hypochondroplasia	AD	*FGFR3*	146000
Leri-Weill dyschondrosteosis (Madelung)	AD	*SHOX or SHOXY*	127300
Metaphyseal chondrodysplasia Jansen type	AD	*PTHR*	156400
Metaphyseal chondrodysplasia Schmid type	AD	*COL10A1*	156500
Multiple epiphyseal dysplasia	AD/AR	*COMP*	132400
Nail-patella syndrome	AD	*LMXB1*	161200
Pseudoachondroplasia	AD/AR	*COMP*	177170
Spondyloepiphyseal dysplasia congenita	AD	*COL2A1*	183900
Spondyloepiphyseal dysplasia tarda	AD/AR	?	184100
Spondyloepiphyseal dysplasia X-linked	X-linked	*SEDL*	313400
Spondylo-metaphyseal dysplasia	AD	*COL2A1*	184250
Stickler syndrome	AD	*COL2A1-COL11A1-COL11A2*	108300-604841-184840
Thanatophoric dwarfism	AR	*FGFR3*	187600
SYNDROMES			
Apert syndrome	AD	*FGFR2*	101200
Crouzon syndrome	AD	*FGFR2*	123500
EEC syndrome (ectrodactyly-cleft palate)	AD	*p63*	129900
Ehlers-Danlos syndrome	AD/AR	*COL5A1-COL5A2-COL1A1*	130000
Greig syndrome	AD	*GLI3*	175700
Holt-Oram syndrome	AD	*TBX5*	142900
Jackson-Weiss syndrome	AD	*FGFR2*	123150
Marfan syndrome	AD	*Fibrillin1*	154700
McCune-Albright polyostotic fibrous dysplasia	AD	*GNAS1*	174800
Oro-facio-digital syndrome	X-linked	*CXORF5*	311200
Pfeiffer syndrome	AD	*FGFR2*	101600
Ulnar-mammary syndrome	AD	*TBX3*	181450
MISCELLANEOUS			
Brachydactyly type A1	AD	*IHH*	112500
Brachydactyly type B1	AD	*ROR2*	113000
Brachydactyly type C	AD	*CDMP1*	113100
Hereditary multiple exostoses type 1	AD	*EXT1*	133700
Hereditary multiple exostoses type 2	AD	*EXT2*	133701
Hereditary multiple exostoses type 3	AD	*EXT3*	600209
Melorheostosis	Sporadic	?	155950
Multiple synostosis syndrome	AD	*Noggin*	186500
Neurofibromatosis	AD	*NF1*	162200
Osteogenesis imperfecta type 1	AD	*COL1A1-COL1A2*	166200
Osteogenesis imperfecta type 2	AD/AR	*COL1A1-COL1A2*	259400-166210
Osteogenesis imperfecta type 3	AR	*COL1A1-COL1A2*	259420
Osteogenesis imperfecta type 4	AD	*COL1A1-COL1A2*	166220
Osteopetrosis congenita	AR	*TCIRG1-CLCN7*	259700
Osteopetrosis tarda	AD	?	166600
Osteopoikilosis	AD	?	166700
Parietal foramina	AD	*MSX2*	168500
Proximal symphalangism	AD	*Noggin*	185800
Pycnodysostosis	AR	*cathepsin K*	265800
Synpolydactyly	AD	*HOXD13*	186300

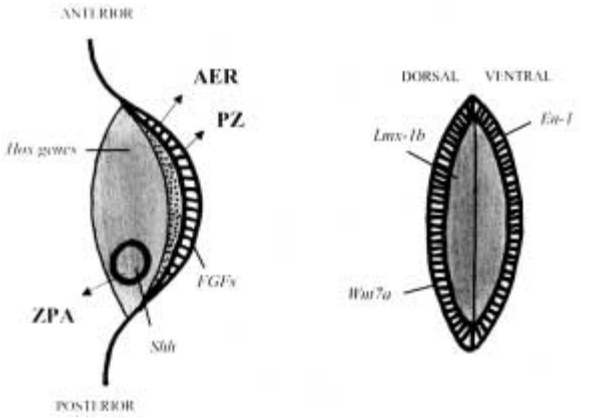

Fig. 2. — Schematic representation of the three major regions in the developing limb bud together with the most important signaling molecules (transverse section at the left, sagittal section at the right). The apical ectodermal ridge (AER), a specialized epithelial structure covering the limb bud, controls the proximo-distal outgrowth of the developing limb and is also involved in dorso-ventral patterning. The zone of polarizing activity (ZPA), a region of posterior limb bud mesenchyme, regulates the antero-posterior patterning. The progress zone (PZ) is the area of undifferentiated mesenchyme below the AER. When cells leave the PZ, they have all the necessary positional information along the three axes.

a) *Disorders of the limbs*

Considerable advances have been made in understanding the molecular mechanisms of vertebrate limb development (21, 34, 49, 60). The outgrowth and development of the limb is a complex process that occurs along three axes : the proximo-distal axis, the antero-posterior axis and the dorso-ventral axis. Patterning along these axes is controlled by different signaling molecules. The key structure in the process of vertebrate limb development is the **limb bud**. Each limb bud is composed of an outer ectodermal cap and an inner mesodermal core. Experiments in the chick embryo have led to the identification of three major regions within the developing limb bud (fig. 2). The **apical ectodermal ridge (AER),** a specialized epithelial structure at the distal tip of the ectodermal jacket overlying the limb bud, regulates the proper formation of structures along the proximo-distal axis of the developing limb. The **zone of polarizing activity (ZPA),** a region of posterior limb bud mesenchyme, is responsible for antero-posterior patterning and the **progress zone (PZ)**, an undifferentiated area of mesenchyme under the AER, receives signals from both the AER and the ZPA. When cells exit the progress zone, they have all the necessary positional information along the three axes. Cells that leave the PZ at an early stage will develop into more proximal structures, whereas cells that leave the PZ at a later stage will develop in more distal structures.

Fibroblast growth factors (FGFs) are important in limb bud initiation and outgrowth. Once formed, further outgrowth of the limb bud is controlled by the AER. Removal of the AER results in distal limb truncations. The level of truncation depends on when the AER is removed : early removals lead to proximal truncations whereas later removals lead to more distal truncations. Sonic hedghog (Shh) mediates the activity of the ZPA and is thus responsible for antero-posterior patterning. *Shh* expression is restricted to the ZPA, which is located in the posterior limb bud. Grafting of Shh expressing cells along the anterior margin of the limb bud results in the formation of ectopic mirror-symmetric digits. Dorso-ventral patterning requires the expression of several other signaling molecules. Wnt7a is only expressed in the dorsal ectoderm and induces the expression of the transcription factor Lmx-lb in the underlying mesoderm. The homeobox-containing transcription factor Engrailed-1 (En-1) is expressed only in the ventral ectoderm.

As mentioned before, the limb bud is the key structure in upper and lower limb development. The outgrowth of the upper limb begins at day 24, whereas outgrowth of the lower limb bud starts at day 28. Morphologically, the differences between the forelimb and hindlimb are very clear. Molecularly, there are differences in the molecules that specify the limb identity. In the developing embryo, limb-specific expression of *Pitx1*, *Tbx4*, and *Tbx5* regulates the determination of limb identity, but there is strong evidence that several other genes also play an important role (60, 41, 27).

All these signaling molecules make sure that cells in the developing limb develop in the correct manner and give rise to a cartilaginous “Anlage”. The “Anlagen” are formed in a proximal to distal sequence : the humeral anlage is formed first, the anlage for the digits is formed last. Joints and fin-

gers are subsequently formed through a process of programmed cell death (apoptosis). Bone morphogenetic proteins (BMPs), cartilage-derived-morphogenetic proteins (CDMPs or growth and differentiation factors (GDFs)), and transcription factors like *HOX* (homeobox) and *T-box* genes play an important role in these processes.

The importance of all these signaling molecules is illustrated by the different congenital malformations in humans and mice, caused by mutations in these genes. Mutations in GLI3, one of the transcription factors involved in the Sonic hedghog pathway, cause Greig cephalopolysyndactyly (skull deformity associated with polysyndactyly of hands and feet ; OMIM 175700), Pallister Hall syndrome (hypothalamic hamartoma, anal defects, variable degree of syndactyly and postaxial polydactyly of hands and feet ; ONIM 146510) or postaxial polydactyly type A (well formed, functional extra digit that articulates with the 5th or extra metacarpal/metatarsal or a pedunculated postminimus (OMIM 174200)) (35, 52, 53, 62). In mice lacking Noggin, cartilage condensations initiated normally but developed hyperplasia, and initiation of joint development failed (6). Human *NOG* mutations were found in families with multiple synostoses syndrome (SYNS 1 ; OMIM 186500) and also in families with proximal synphalangism (SYM1 ; OMIM 185800) (27). Both SYM1 and SYNS1 have multiple joint fusion as their principal feature. Other examples are the mutations in *HOXD13* which cause synpolydactyly (SPD ; OMIM 186300), characterized by syndactyly between the third and fourth fingers, with digit duplication in the syndactylous web combined with syndactyly of the fourth and fifth toes. Mutations in *CDMPI* cause brachydactyly type C (OMIM 113100) (50). Here, the anomalies of the digits are of many types : brachydactyly of the middle phalanges of the index and middle fingers, triangulation of the fifth middle phalanx, brachymetapody, hyperphalangy (more than 3 phalanges per finger), symphalangism. Holt-Oram syndrome (OMIM 142900), caused by mutations in one of the T-box genes, *TBX5*, is characterized by limb anomalies ranging from triphalangeal thumb to complete absence of the limb, together with cardiac malformations (4). On the other hand, mutations in *TBX3*, are responsible for the ulnar-mammary syndrome (OMIM 181450) (2, 3). Here, the limb malformations range from duplication of the fifth digit to complete absence of hand and forearm. Additional features of this syndrome include defects of the teeth, genitals and apocrine glands including the breasts. Mutations in *MSX2*, another homeobox containing transcription factor, have been described in families with autosomal dominant craniosynostosis and in patients with cranial defects of the parietal bones (foramina parietalia permagna ; OMIM 168500) (33, 63). EEC syndrome (OMIM 129900) is an autosomal dominant disorder characterized by ectrodactyly, ectodermal dysplasia, and facial clefts. Recently, Celli *et al*. (10) found that this syndrome is caused by mutations in the *p63* gene. Mutations in this gene are also responsible for the split hand/split foot malformation (OMIM 605289) (32). Mutations in *LMX1B* are responsible for the patella-nail syndrome (OMIM 161200) which is characterized by dysplasia of the nails and absent or hypoplastic patellae and in some cases nephropathy (20).

b) *Disorders of the axial skeleton*

Ribs and vertebrae are frequently involved in malformation syndromes. Both originate from the sclerotome, a structure composed by differentiation of the somites. Somites are blocks of epithelial cells and they give rise to the development of ribs and vertebrae, the dermis of the dorsal skin, and the skeletal muscles of the body wall and limbs (5, 19, 28). The Notch-Delta pathway is crucial in somitogenesis as demonstrated by several knock-out mutations *(Mesp2, Notch1, Dll1, lunatic fringe* and *Dll3*) in mice. Recently, mutations in the human homologue DLL3 were found to cause axial skeletal defects in spondylocostal dysostosis (SD ; OMIM 277300), a heterogenous group of disorders characterized by multiple hemivertebrae, rib fusions and deletions with a non-progressive kyphoscoliosis (9).

c) *Disorders affecting the formation and growth of bone and cartilage*

Undifferentiated cells condensate according to a pattern that outlines the future skeletal elements

('Anlagen'). After condensation, these precursor cells differentiate into chondrocytes or osteoblasts and produce their own specific extracellular matrix (ECM). Bones then develop either through a process of intramembranous ossification (clavicle, flat bones of the skull and the mandible) or through a process of endochondral bone formation (a temporary cartilaginous template is subsequently replaced by bone). Mutations in *RUNX2,* a gene involved in intramembranous ossification, lead to cleidocranial dysplasia (OMIM 119600) (46). Further longitudinal bone growth is achieved in the growth plate of long bones through the proliferation and differentiation of chondrocytes. The growth plate consists of several zones and each zone is under the specific control of regulatory genes (13). In this way, a tight control of proliferation, differentiation, ECM production and cartilage removal is maintained. Several genes involved in these processes have been identified. *FGFR3* keeps the chondrocytes undifferentiated in the resting zone of the growth plate. Mutations in *FGFR3* cause achondroplasia (characterized by a long, narrow trunk, short extremities, particularly in the proximal (rhizomelic) segments, a large head with frontal bossing, hypoplasia of the midface and a trident configuration of the hands ; OMIM 100800), hypochondroplasia (similar as achondroplasia but no involvement of the head ; OMIM 146000), and thanatophoric dysplasia (lethal form of dwarfism ; OMIM 187600) (51, 56, 58). Differentiation of proliferating chondrocytes in the transition zone into hypertrophic cells is controlled by Indian hedghog and PTHrP. Mutations in the gene encoding Indian hedgehog are responsible for brachydactyly type Al (OMIM 112500) (25). In this type of brachydactyly, the middle phalanges of all the digits are rudimentary or fused with the terminal phalanges. In the lower part of the hypertrophic zone of the growth plate, chondrocytes produce collagen into their ECM which starts to calcify. The calcified cartilaginous matrix is removed by osteoclasts and replaced by bone. At the end of the normal growth period the growth plate is completely replaced by bone. The major components of the ECM of normal cartilage are aggrecan and collagen type II. Collagen type I is the major component of bone, tendons and perichondrium. Mutations in *COL2A1*, the gene encoding type II collagen, can result in several types of dysplasia (achondrogenesis (OMIM 200610), Stickler syndrome (OMIM 108300), spondyloepiphyseal dysplasia congenita (OMIM 183900). Osteogenesis imperfecta types I-IV (OMIM 166200/166210/166220/259420) is caused by mutations in *COL1A1* and *COL2A2*. Multiple epiphyseal dysplasia type 1 (OMIM 132400) and pseudoachondroplasia (OMIM 177170) are caused by mutations in *COMP* (cartilage oligomeric protein), one of the non-collagenous proteins in the ECM (6).

Apart from the collagen and the non-collagenous proteins the ECM of chondrocytes also contains sulfated proteoglycans. Defects in the biochemical pathways that lead to sulfation of these proteoglycans result in distinct chondrodysplasias like diastrophic dysplasia (OMIM 222600) caused by mutations in the gene encoding a sulphate transporter (*DTDST*) (31).

d) *Disorders caused by abnormal matrix homeostasis*

A continuous interaction between osteoclasts (bone resorption) and osteoblasts (deposition of new bone matrix) results in normal bone turnover and remodelling. In osteopetrosis or marble bone disease (OMIM 259700) there is more bone production than bone resorption. As a consequence of this, very dense bone without trabeculation is produced and bone marrow becomes replaced by bone, which may result in aplastic anaemia and death. Frattini *et al.* (22) and Kornak *et al.* (37) showed that *TCIRG1*, encoding the osteoclast-specific 116-kD subunit of the vacuolar proton pump, was mutated in 5 of 9 patients with infantile malignant osteopetrosis. Recently Kornak *et al.* (36) demonstrated that loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. Pycnodysostosis (OMIM 265800), a disorder characterized by deformity of the skull (including wide sutures), maxilla and phalanges (acroosteolysis), osteosclerosis, and fragility of bone, is caused by mutations in the lysosomal enzyme cathepsin K (26). In osteoporosis bone catabolism exceeds bone

anabolism, resulting in a generalized reduction in bone mass. Polymorphisms in the *COL1A1* gene, the *IL-6* gene and in the genes for vitamin D and the calcitonin receptor have been associated with osteoporosis (29, 40, 48, 54). Several mouse mutants exist that display osteoporosis as part of their phenotype. For instance, mice deficient for the proteoglycan biglycan show a reduced growth rate and decreased bone mass after birth (64).

This overview is certainly not meant to be exhaustive, since almost every week new scientific data from the field of skeletal development are published. It merely illustrates that many orthopedic conditions have a genetic basis and that science is progressively unraveling the underlying causes. How can all this information be of any use in daily orthopedic practice ? Currently one of the limiting factors to assign a gene to a disease is the availability of patients with these diseases. The orthopedic surgeon finds himself in a privileged position since he is often one of the first to see patients with rare genetic diseases. Moreover he can provide scientists with clinical samples like articular cartilage, synovial tissue, bone, skin for further research. Since abnormal genes are the basis of many orthopedic disorders, ranging from skeletal dysplasias to osteoarthrosis, orthopedic surgeons need to be aware of the data already available and of the increasing technical possibilities in molecular biology. As will be demonstrated, understanding the genetic basis of inherited orthopedic malformations can help in making a correct diagnosis and counsel patients and families. Eventually, it will also provide the orthopedic surgeon with new therapeutic tools. Finally, the identification of individuals or patients with unique skeletal malformations will help scientists to gain more insight into the complex mechanisms of skeletal development.

Diagnosis of inherited skeletal malformations and counseling

When confronted with a congenital malformation, making the correct diagnosis is a first and important step in the further management of the patient. Making a correct diagnosis is not only helpful in determining the normal evolution, the mode of inheritance and prognosis of the disorder, but will also aid in choosing the appropriate treatment. In order to make a correct diagnosis several classification systems have been proposed. Usually classification systems for congenital orthopedic malformations are based on the clinical and radiological appearance. In the past, various Greek and Latin names were adopted to describe common deficiencies (for example acheira, amelia, ectromelia, micromelia, peromelia, phocomelia,...). These terms often were very confusing to most clinicians. Therefore many authors have tried to develop a more useful classification system based on defects in normal embryologic development. In 1968, Swanson, Barsky and Entin proposed their work "Classification of limb malformations on the basis of embryological failures" (57). In this work, deformities were grouped according to the parts that have been primarily affected by certain embryological failures. The International Federation of Societies for Surgery of the Hand (IFSSH) proposed seven categories based upon the original classification of Swanson. Despite its usefulness, this classification system is not the ideal one as demonstrated by Buck- Gramcko (8) and De Smet *et al*. (15). Other classification systems, like the one described by Temtamy and McKusick (59), are based on anatomical and genetic grounds. This classification system is still used by most clinicians. The use of classification systems based on clinical appearance and skeletal radiology is frequently complicated by a large variation in expressivity and considerable overlap between apparently different malformation syndromes. Further understanding of the molecular pathways involved in normal skeletal and limb development will lead to an entirely different classification system wherein the affected gene/or pathway can be associated with its phenotypical spectrum. For instance, Greig cephalopolysyndactyly, Pallister Hall syndrome and postaxial polydactyly type A1 are all caused by mutations in the *GLI3* gene, but clinically and radiologically these three syndromes are very distinct. When using the 'classical' classification systems they would fall into three separate categories whereas molecularly they fall into one single category.

Inherited skeletal malformations are often associated with other visceral malformations. Mutations in *TBX5* for instance, cause Holt-Oram syndrome. This syndrome is not only characterized by hand malformations but is also associated with cardiac malformations. In these cases, counseling may not be limited to determining the possible recurrence risk of the limb deformity, but it must also include a screening for possible associated cardiac malformations. Recently, we identified a girl with a complex type of synpolydactyly associated with urogenital problems. She was diagnosed as having Hand-Foot-Genital-Syndrome (HFGS) associated with synpolydactyly (SPD). Three other family members on the maternal side of the family were also known to have SPD. In HFGS, (OMIM 140000) there is first digit and hallux hypoplasia, brachydactyly of second to fifth toes, clinodactyly of the fifth finger and ulnar deviation of the second finger. Males often have hypospadias. Müllerian duct fusion defects in females result in a vaginal septum, a double uterus with double cervix, and urinary abnormalities (ectopic ureteric orifices, vesico-ureteral reflux, pelvi-ureteric junction obstruction). SPD is caused by mutations in the *HOXD13* gene whereas mutations in the *HOXA13* gene cause HFGS. Mutation analysis in this patient revealed both mutations in *HOXD13* and *HOXA13* (Debeer *et al.*, manuscript in preparation). Further analysis within this family showed that the *HOXD13* mutation was present in all affected members on the maternal side. The *HOXA13* mutation came from the paternal side but HFGS was not previously diagnosed here. Upon further investigation subtle urogenital problems were identified in this side of the family. This example illustrates how genetic investigation of an orthopedic malformation clearly has implications in counseling other family members.

Therapeutic and scientific implications

Congenital orthopedic malformations provide a unique opportunity to identify novel molecules involved in normal skeletal development. The identification of such molecules offers potential for new therapeutic strategies in several orthopedic conditions (55). Recombinant human OP-1 (BMP7) is already being used in clinical trials in the treatment of tibial nonunions (23, 24). Increasing knowledge of the molecular pathways involved in the normal differentiation of osteoblasts and chondrocytes will allow us to modify progenitor cells so that they specifically produce the desired molecules when administered to the patient, for example for the treatment of cartilage defects. Genes directly involved in skeletal development can be used as drug targets or as reagents for drug development. For example, drugs that inhibit osteoclast activity could be very valuable in treating patients with Paget's disease or osteoporosis. The discovery of novel molecules or the attribution of novel functions to already known molecules will also have its effect on orthopedic tissue engineering (for a review on tissue engineering see Clin. Orthop. 1999 Oct ; 367 Suppl.) and on the possible applications of gene therapy in certain orthopedic disorders (30).

The following case is an illustration of how an orthopedic malformation can lead to the discovery of molecules possibly involved in normal limb development. All affected members in a family with a complex type of synpolydactyly associated with synostoses were found to have a balanced translocation t(12;22)(p11.2; q13.3) between chromosome 12 and chromosome 22 (14, 16). The cosegregation of the phenotype with the translocation lead to the hypothesis that on one or on both chromosomes a gene was located involved in normal limb development. When disrupted by the translocation, it would give rise to the observed limb phenotype. Detailed molecular analysis of this translocation resulted in the identification of two genes directly involved in the translocation (17, 18). One of these genes, *Fibulin-1* turned out to be the ideal candidate for the phenotype since it is expressed in the developing handplate. We were able to demonstrate that fibroblasts in the synpolydactyly patients produced significantly less Fibulin-1D protein as compared to normal skin fibroblasts. This finding supports the hypothesis that haploinsufficiency for Fibulin-1D is the cause for this limb malformation and illustrates once more the importance of a normal ECM in skeletal development.

CONCLUSION

Orthopedic surgeons need to be aware of the current advances and possibilities in molecular biology and genetics. The increasing amount of information regarding skeletal development has not only diagnostic but also possible therapeutic implications. A good communication between orthopedic surgeons, molecular biologists and geneticists is therefore essential.

GLOSSARY

Haploinsufficiency : describes the case where a 50% reduction in the level of gene function causes an abnormal phenotype.

Homeobox : conserved DNA sequence that encodes a DNA-binding motif famous for its presence in genes that are involved in orchestrating development of a wide range of organisms.

Knock-out mutation : the targeted inactivation of a gene in an intact cell.

Linkage : the tendency of genes or other DNA sequences at specific loci to be inherited together as a consequence of their physical proximity on a single chromosome.

Locus : unique chromosomal location defining the position of an individual gene or DNA sequence.

Polymorphism : any sequence variant present at a frequency > 1% in a population or any nonpathogenic sequence variant, regardless the frequency.

Transcription factor : eukaryotic protein required to initiate or regulate transcription (= copying one strand of DNA into a complementary RNA sequence by the enzyme RNA polymerase).

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SAMENVATTING

PH. DEBEER, L. DE SMET, W. J. M. VAN DE VEN, G. FABRY, J.-P. FRYNS. Genes and orthopedics : from gene to clinic and vice versa.

De recente ontwikkelingen binnen de genetica en de moleculaire biologie zorgen voor een toenemende kennis van de moleculaire mechanismen verantwoordelijk voor een normale skeletontwikkeling. Verschillende genen die hierbij betrokken zijn werden reeds geidentificeerd, doch vele moeten nog ontdekt worden. Mutaties in dergelijke genen zijn vaak de oorzaak van de congenitale skeletafwijkingen welke gezien worden binnen de orthopedie. In dit overzichtsartikel willen we het belang van een goede interactie tussen orthopedisten, genetici en moleculair biologen benadrukken.

RÉSUMÉ

PH. DEBEER, L. DE SMET, W. J. M. VAN DE VEN, G. FABRY, J.-P. FRYNS. La génétique et l'orthopédie : du gène à la clinique et vice versa.

Les progrès récents de la génétique et de la biologie moléculaire ont beaucoup contribué à une meilleure compréhension des mécanismes qui sont impliqués dans la morphogenèse normale du squelette. On a identifié de nombreux gènes impliqués dans le développement normal du squelette ; de nombreux autres gènes restent à découvrir. Des mutations au niveau de ces gènes sont souvent à l'origine de malformations congénitales du squelette, que les orthopédistes rencontrent en clinique. Dans cette revue générale, les auteurs soulignent l'importance d'une interaction entre chirurgiens orthopédiques, biologistes moléculaires et généticiens.