Multiple Epiphyseal Dysplasia
A Clinical and Molecular Genetic Study

Multipele epifysaire dysplasie
Een klinische en moleculair genetische studie

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit te Rotterdam
op gezag van de Rector Magnificus
Prof.dr P.W.C. Akkermans M.A.
en volgens besluit van het college voor promoties

De openbare verdediging zal plaatsvinden op
9 september 1998 om 15.45 uur

door

Johannes Bernardus Antonius van Mourik

geboren te Gorinchem

Promotiecommissie

Promotor	:	prof.dr J.A.N. Verhaar
Overige leden	:	prof.dr S.L.S. Drop
		prof.dr M. Meradji
		prof.dr M.F. Niermeyer
		dr E.C.M. Mariman (tevens co-promotor)

ISBN 90-9011789-X

This publication was financially supported by grants from the Nederlandse Orthopaedische Vereniging and the Sint Joseph Ziekenhuis, Veldhoven.

Voor allen die mij dierbaar zijn.

Publications and papers based on studies in this thesis

Een Nederlandse familie met erfelijke gewrichtsklachten: multipele epifysaire dysplasie.
J.B.A. van Mourik, C. Schaap en A.J.G. Nollen.
Ned Tijdschr Geneeskd 1993;137:32-36.

A mutation in the gene encoding the α2 chain of the fibril-associated collagen IX, COL9A2, causes multiple epiphyseal dysplasia (EDM2).
Yasuteru Muragaki, Edwin C.M. Mariman, Sylvia E.C. van Beersum, Merja Perälä, Jan B.A. van Mourik, Matthew L. Warman, Bjorn R. Olsen & Ben C.J. Hamel.
Nature Genetics 1996;12:103-105.

A mutation in COL9A2 causes multiple epiphyseal dysplasia (EDM2).
Yasuteru Muragaki, Edwin C.M. Mariman, Sylvia E.C. van Beersum, Merja Perälä, Jan B.A. van Mourik, Matthew L. Warman, Ben C.J. Hamel, Bjorn R. Olsen.
Ann N Y Acad Sci 1996;785:303-306.

Radiographic Anthropometry in Patients with Multiple Epiphyseal Dysplasia.
Jan van Mourik, Hans Weerdenburg.
AJR 1997;169:1105-1108.

A Large Family with Multiple Epiphyseal Dysplasia Linked to COL9A2 Gene.
Jan B.A. van Mourik, Ben C.J Hamel and Edwin C.M. Mariman.
Am J Med Genet 1998;77:234-240.

Electron Microscopical Study in Multiple Epiphyseal Dysplasia type-II.
Jan B.A. van Mourik, Pieter Buma, William R. Wilcox.
Ultrastr Path (in press).

MRI in Multiple Epiphyseal Dysplasia: No Additional Value for Diagnosis and Treatment.
J.B.A. van Mourik, J.P.G. Weerdenburg, J.A.N. Verhaar.
(submitted).

Radiographic Anthropometry In Patients with Multiple Epiphyseal Dysplasia.
J.B.A. van Mourik, J.P.G. Weerdenburg.
Poster presentation, The 3rd International Skeletal Dysplasia Meeting, California, August 7-9, 1997.

Contents

Introduction

Multiple epiphyseal dysplasia (MED) is one of the most common osteochondrodysplasias [Wynne-Davies and Gormley 1985]. During childhood and adolescence it affects the epiphyses of the tubular bones, resulting in axial deformities and shorter limbs. Later in life MED can lead to osteoarthritis. The disease has been given its name by Sir Thomas Fairbank in 1947 [Fairbank 1947].
The diagnosis is based on physical examination and radiological survey. Radiographic abnormalities appear after the third year of life. The most important finding is the delay and irregularity of ossification of the involved epiphyses. The epiphyses of all long bones can be affected. Radiographic anthropometric studies have been performed, being of value in early diagnosis of MED in case of doubt [Poznanski et al. 1972, Ingram 1992]. The disease is inherited and affects both sexes. The type of inheritance is autosomal dominant.
Histology of cartilage of patients with MED reveals paucity of chondrocytes in all zones of the growth plate as well as loss of orientation of the chondrocytes [Anderson et al. 1962]. These changes, however, are similar to those seen in other osteochondrodysplasias. Electron microscopical studies have shown dilations of rough endoplasmic reticulum containing a material with alternately wide electron dense and electron lucent layers [Stanescu et al. 1993].
Treatment is symptomatic. In case of limited range of motion physical therapy and nonsteroidal anti-inflammatory drugs may be prescribed; in case of axial deformities osteotomies can be performed *(Chapter 1)*.

Although MED is a well known osteochondrodysplasia, it has a low prevalence of 11 to 16 per million. At the Department of Orthopedic Surgery of the Sint Joseph Ziekenhuis in Eindhoven an unexpectedly high number of patients with MED was seen. This gave us the opportunity to study MED in detail. Therefore, we started a research project on these patients, especially concerning the heredity. Based on clinical features and radiographic findings, a pedigree could be established, which is the largest ever described *(Chapter 2)*.
In collaboration with the Department of Human Genetics of the University Hospital Nijmegen a study was performed to detect the chromosomal location of the genetic defect in the studied family *(Chapter 3)*. After determination of the locus, RNA from chondrocytes was investigated in order to search for a mutation in a candidate gene, the gene for COL9A2 *(Chapter 4)*. This study was performed in collaboration with the Departments of Cell Biology of Boston, USA, Medical Biochemistry of Turku, Finland and the Department of Genetics Cleveland, USA.
With radiographic anthropometric studies, it might be possible to quantify the degree of dysmorphogenesis, and to detect the affected members of a lineage with a skeletal dysplasia. The sensitivity, specificity and positive predictive values of these measure-

ments for having MED were analysed *(Chapter 5)*. The value of MRI in the diagnosis of MED was determined *(Chapter 5)*. Some patients of this family were operated on for various reasons; the macroscopic findings at these operations were studied, as well as the light-microscopical and electron-microscopical findings of specimens obtained at operation *(Chapter 6)*.

In summary, the aims of this thesis were:

- To establish a pedigree with a clear clinical distinction between patients and unaffected relatives.
- To localize the gene involved in MED in the studied family.
- To identify the exact molecular defect for MED.
- To study the usefulness of anthropometric studies and MRI in this family.
- To study the morphology of the affected cartilage.

1 Survey of the literature

1.1 Introduction

Multiple epiphyseal dysplasia (MED) belongs to the group of osteochondrodysplasias; it is an hereditary disease. It affects the epiphyseal ends of the long bones, the carpal and tarsal bones, and sometimes the vertebrae. Onset of the disease is in early childhood and the disease is characterised by pain and stiffness of the joints. The exact cause is not known; the prevalence is low, probably about 11 to 16 per million.

1.2 Osteochondrodysplasias

Osteochondrodysplasias or skeletal dysplasias are diseases affecting growth of bone and cartilage. They are often inherited and can affect other organ systems, causing for example deafness, blindness or mental retardation. The clinical expression is variable. Some skeletal dysplasias are lethal in the prenatal period, others are diagnosed at birth. Some have their onset in infancy, childhood or young adult age [Shapiro 1987]. In these later manifesting cases, pediatricians or orthopaedic surgeons are usually consulted because of growth retardation. This shortness of stature can be proportionate or disproportionate. In proportionate shortness the limbs and trunk are relatively proportionated in size as in normal individuals. Children with this type of growth retardation ('midgets') are investigated by pediatricians for endocrine or chronic medical diseases. In disproportionate shortness the limbs are often shorter than the trunk ('dwarfs') [Lovell and Winter 1986], and these children are mostly seen by orthopaedists.
Fairbank proposed the first classification for the skeletal dysplasias in 1951, and divided them into three groups: those with short limbs, those with short trunks and proportionate midgets. Rubin [1964] suggested a classification based on the nature of any of three bone modeling errors: dysplasias, dystrophies and dysostoses.
Dysplasia means wrong or disturbed formation. The predominant error is in intrinsic bone modeling. It affects all growing bones similarly and is generalized in distribution. The resulting pathophysiologic defect can lead to either aplasia, hypoplasia or hyperplasia.
Dystrophy means 'ill nourished': a disease caused by defective and faulty metabolism and nutrition. The causative error is thought to be extrinsic to bone, and the resulting disease may affect all bones or be selective, depending upon the distribution of the primary defect.
Dysostoses are the abnormalities in bone modeling secondary to changes in soft tissues, muscles and nerves. Here, the distribution of skeletal defects is not pre-

dictable, because they follow the primary defect in ectodermal or mesenchymal tissues.
Rubin's *Dynamic Classification* differentiates for areas of bone involvement: (1) epiphyseal, (2) physeal (the area of the growth plate), (3) metaphyseal and (4) diaphyseal [Rubin 1964]. This classification became very helpful, but was not generally accepted. Terms like dysplasia or dysostosis were differentially applied with resulting confusion.
Apley and Solomon [1993] classified also on the localisation of the bone area involved, but in a more functional approach. They recognise four categories: (1) predominantly physeal and metaphyseal changes; (2) predominantly epiphyseal and/or vertebral body changes; (3) mainly diaphyseal changes; and (4) various combinations of epiphyseal, metaphyseal, vertebral and cranial abnormalities.
In 1969 the European and North American Societies for Pediatric Radiology accepted the Paris Nomenclature of Skeletal Dysplasias [Kaufmann 1976, Tachdjian 1990]. This classification is very extensive and according to some too difficult for general use. However, this is the classification most used, and it is adjusted every two years (Chapter 10).
Basically, accepting the poorly differentiating value of dysplasia, dystrophy or dysostosis, the zonal localisation-classification is useful for clinical and radiological differentiation. In the future, insight into molecular mechanisms may allow a completely different type of classification, based upon either basic morphogenetic mechanisms, or genetic defects in major structural proteins involved in skeletal development.

1.3 Genetics

Genetic factors play a dominant role in skeletal dysplasias. Genetic defects are present from conception onwards, and may severely affect development from pre- to postnatal life. Genetic defects can be divided into chromosomal, single gene or multifactorial disorders.
The position of a gene on a chromosome is called a locus. At each locus there are two forms of a gene, the alleles, as chromosomes are paired. One allele is paternal, the other is maternal. If the two alleles are identical, the person is said to be homozygous; if not, the person is heterozygous.
Single gene disorders are caused by mutations in one gene, or in a pair of alleles. Types of mutations differ from insertion, deletion, substitution, inversions, etc. There are thousands of these single gene disorders transmitted by the rules of Mendelian inheritance, and they are summarized in McKusick's Mendelian Inheritance in Man [1994]. These monogenic or single gene disorders are classified as autosomal dominant/-recessive, X-linked dominant/-recessive, or mitochondrial, based upon the location of the genetic defect, and its expression in homozygous (double dose) or heterozygous (single dose) status.
In autosomal dominant inheritance the affected parent is heterozygous and has an abnormal and a normal gene for the specific condition. The children have a 50% chance to inherit the disease. The pedigree shows a vertical pattern of inheritance: in subsequent generations, the disorder is transmitted by an affected individual (male

or female) to part (50%) of the offspring. Often, the disease can be variable among affected individuals. In general, the abnormal gene manifests itself in the phenotype, irrespective of the presence of the normal allele [Thompson et al. 1991].
In autosomal recessive inheritance, a patient receives an abnormal gene from both parents, and the recurrence risk to siblings of a patient is 1:4 (25%) for a similar disorder. Usually, the disorder is only present among siblings of affected patients, and only rarely among the relatives.
In X-linked disorders, the gene involved is located on the X-chromosome. Male patients with X-linked gene mutations are usually severely affected, while heterozygous females are either healthy or show moderate symptoms. In subsequent generations, males are affected, with intervening (healthy) females.
The apparently simple Mendelian rules often cannot explain nature's variability. Genetic disorders may differ in pattern of inheritance (both dominant and recessive forms of a skeletal dysplasia known), or there are environmental factors hypothesized. Also, multifactorial causes (combinations of genetic and non-genetic factors) may be involved. Genetic heterogeneity indicates that a certain phenotype may be caused by different gene mutations on the same locus (allelic heterogeneity) or by mutations on different loci (locus heterogeneity). All these causes of heterogeneity are known among the skeletal dysplasias [Dietz and Mathews 1996].

1.4 Diagnosis of Skeletal Dysplasias

Reasons for referral in skeletal dysplasias are generally shortness of stature, deformities of the long bones or skull and pain or fractures. The family history and physical examination will usually guide the medical work-up. The family history is very relevant, especially in cases of autosomal dominant (one parent or parent's relatives affected), X-linked (male maternal relatives affected) skeletal dysplasias, and sometimes in autosomal recessive cases (previous siblings affected).
Deformities may be angular in limbs, like genu varum or valgum, broadness of the epiphysis, shortness of limbs or trunk, numerical or grossly structural like polydactyly and syndactyly, contracture of digits, and abnomalities of skull and ears. Pain is frequent in metabolic bone diseases (the affected bones are painful on palpation) and in elderly patients with bone dysplasia resulting in osteoarthritis with pain in the affected joints.
Measuring standing and sitting height is important to differentiate between proportionate or disproportionate shortness. The span of the upper limbs may be compared with the standing height to establish excessive span or shortening of limbs. The face and skull must be inspected, the shape of the nose and nasal bridge (achondroplasia), the hair (trichorhinophalangeal syndrome) and skin. Eyes and teeth may give important clues (osteogenesis imperfecta) as well as the ears (dystrophic dysplasia). The spine is inspected for deep lumbar lordosis (achondroplasia) or scoliosis. Limbs, hands and feet will be inspected and measured for angular deformities, contractures, shortness of metacarpals and deformities of fingers and toes.
Radiographic studies are essential. Tachdjian [1990] advised eight projections: (1) lateral projection of the skull; (2) anteroposterior and (3) lateral of the entire spine;

(4) anteroposterior of the thoracic cage with clavicles and shoulders; (5) anteroposterior of the pelvis with hips and symphysis pubis; anteroposterior of (6) one knee and (7) one forearm, including elbow and wrist; and (8) posteroanterior projection of one hand, including all metacarpals and digits. Others, like Apley and Solomon [1993] suggested an approach with three radiographs as sufficient in most cases: an anteroposterior of the pelvis and hips, an AP of the wrists and hands and a lateral projection of the spine.
The need for laboratory studies evolves with increasing understanding of the genetic causes of skeletal dysplasias. Classical clinical work-up included plasma calcium, inorganic phosphate, alkaline phosphatase, magnesium and sometimes other metals. A urinary metabolic screen requires analysis of excretion of calcium, creatinine and hydroxyproline, but also of amino acids, mucopolysaccharides, oligosaccharides, and organic acids. Occasionally a bone biopsy is helpful. If the differential diagnosis includes a skeletal dysplasia caused by a known gene mutation, DNA-studies of the index patients, the parents or other relatives are indicated. This specifically applies to disorders caused by mutations in collagen genes, Fibroblast Growth Factor receptor genes, and an increasing number of others.

1.5 Multiple Epiphyseal Dyplasia

1.5.1 History

Multiple epiphyseal dysplasia (MED) belongs to the group of dysplasias with predominantly epiphyseal changes. The first International Nomenclature of Constitutional Disease of Bones classifies it as an osteochondrodysplasia, with defects of growth of tubular bones and/or spine, identifiable in later life [Kaufmann 1976]. According to the International Classification of Osteochondrodysplasias, second revision, MED belongs to the defects of the tubular (and flat) bones and/or axial skeleton *(Chapter 10)*.
The earliest report of MED is probably that of Barrington-Ward [1912], describing peripheral joint involvement in a brother and sister. Later cases of irregular ossification of several epiphyses were reported under a variety of diagnoses [Silfverskiöld 1925, Silfverskiöld 1926, Grudzinski 1928, Clark 1929, Bettman 1932, Jansen 1934, Hirsch 1937, Müller 1939A, Müller 1939B]. Sir Thomas Fairbank [1935], describing epiphyseal skeletal problems, suggested the name 'dysplasia epiphysealis generalisata', but he exchanged the term 'generalisata' for 'multiplex' as being more accurate. Two of such cases were presented to the Royal Society of Medicine [Wiles 1938, Yarrow 1938]. Fairbank [1946] collected 15 cases and suggested that MED was a developmental error of unknown cause, characterized by dwarfism, stubby digits, and mottling or irregularity in density and outline of several of the developing epiphyses [Fairbank 1946]. These observations resulted in his often cited lecture on 'Dysplasia Epiphysealis Multiplex' [Fairbank 1947].
A milder form of MED had been observed at the same time on the continent, designated as 'Hereditäre multiple Epiphysenstörungen' [Ribbing 1937]. Since 1947, there has been a wide interest in MED, leading to reports on Fairbank disease, Ribbing disease, Ribbing-Müller disease, achondroplasia atypica, chondro-osteo-dystrophy,

dysostosis epiphysealis multiplex, dysplasia polyepiphysaire, dystrophie osteochondrale polyepiphysaire, and polyosteochondritis. Some of these cases, originally described as MED, later had to be reclassified with other diagnoses [Goetsch 1953, Rennell and Steinbach 1970].
Moreover, the differentiation was proposed between the 'Ribbing type': the milder form of MED with normal hands and wrists, and the 'Fairbank type': with more serious abnormalities, stubby hands and feet [Wynne-Davies and Fairbank 1976]. Since the detection of two loci for MED [Oehlmann et al. 1994, Briggs et al. 1994] the names MED type I and II are used.

1.5.2 *Clinical Features*

MED affects children and adolescents of both sexes. Patients complain about difficulty in walking and pain and stiffness of the knees and hips. At a later age, arthritic symptoms occur. Short stature may be caused by shorter limbs. The hands are striking due to short, thick and stubby fingers and thumbs. The joints originally found involved [Fairbank 1947] are the shoulders, elbows, wrists, hands, hips, knees and ankles. The spine is usually normal; only one case of platyspondyly was seen. Joint stiffness, pain, limp and waddling gait is usually described [Tachdjian 1990, Apley and Solomon 1993, Lovell and Winter 1986]. The disorder is limited to mainly skeletal and joint symptoms. Mental and muscular development is normal, and there are no neurological signs. Children may present with pain in the joints and difficulty in running and climbing stairs. The trunk is of normal height, but the limbs are shorter. Flexion contractures of knees and elbows are frequent, as are genu varum or valgum. These complaints often diminish in adolescence. However, degenerative arthritis may be the manifesting symptom in the hip, knee or ankle in adolescence or early adult life. The face, skull and spine are normal. There is substantial variation in the severity of MED, even among members of the same family.
MED mostly affects bones and cartilage [Jacobs 1968, Mansoor 1970, Short et al. 1979, Brunzlow et al. 1988, Sensenbrenner 1974, etc.]. Less often, associated abnormalities were reported in the eyes or ears [Beighton et al. 1978, Pfeiffer et al. 1973, Walker 1969, Daentl et al. 1975]. Other incidental associations included os trigonum syndrome [Molay et al. 1982]; Turner syndrome [Lowry and Wood 1977]; endocrine abnormalities [Wolcott and Rallison 1972]; or rhizomelic shortness, cleft palate and micrognathia [Lowry et al. 1996]. MED was also reported in combination with other malformations or genetic conditions like the Apert syndrome [Hunter and MacDonald 1989, Cohen and Kreiborg 1993], or unusual vertebral abnormalities [Felman 1969]. The precise classification of MED in all these reports is often difficult, also because of diagnostic confusion with Perthes' disease and other disorders [Wenger and Ezaki 1981] (see below).

1.5.3 *Epidemiology*

There is a widely different perception of the frequency of MED [Lovell and Winter 1986, Tachdjian 1990], and the only prevalence data (4 per 100.000 in Denmark)

[Andersen and Hauge 1989] do not completely support the estimate of Wynne-Davies et al. [1985] of 11 index patients per million, and 16 total cases including affected relatives per million.

1.5.4 *Radiographic Features*

The radiographic abnormalities may appear after the third year of life and are always bilateral. Delay and irregularity of ossification of the involved epiphyseal ends of the long bones and ossification centers for carpal and tarsal bones are most prominent. They ossify from multiple centers and the secondary centers are small in size [Silverman 1985, 1996]. The epiphyses are flattened, mottled and fragmented. The epiphyses of the distal femur and proximal tibia are often broad and very irregular, which may explain the development of genu varum or valgum. The patella may be involved and sometimes subluxation or double layering of the patella is seen [Hodkinson 1962, Coates et al. 1992]. In older children fusion of epiphyseal centers is delayed. Sometimes mild flaring of the metaphysis occurs, but in case of gross metaphyseal changes the diagnosis should be changed to metaphyseal or epimetaphyseal dysplasia [Kozlowski and Budzinska 1966, Resnick 1995]. Metacarpals, metatarsals and phalanges may be short, with small, broad phalanges in hands and feet, with irregular epiphyseal and nonepiphyseal ends of the bones. The secondary centers of the vertebrae may be irregular because of notching and poor development of the ring epiphysis, and sometimes there may be slight anterior wedging of the dorsal vertebrae. This should be differentiated from platyspondyly or spondyloepiphyseal dysplasia. The facial bones and skull are always normal.
At adult age, the articular surfaces of the long bones are irregular and abnormal in shape. The femoral heads and condyles and talar articular surfaces may be flattenend. The proximal end of the tibia is square instead of biconcave. Differential growth within an epiphysis may induce wedge-shaped or V-shaped deformities of the wrist, and tibiotalar slant, and secondary degenerative joint diseases. Osteoarthritis in the hip of a patient with MED is related to retarded development of the hip; in case of a fragmented and flattened ossific nucleus and acetabular dysplasia, osteoarthritis often developed. In case of a rounded, uniformly ossified nucleus of the hip in childhood, the hips did not show osteoarthritis at adult age [Treble et al. 1990].
Bone imaging with technetium-99m usually does not show a lack of uptake as it does in Perthes' disease [Tachdjian 1990]. MacKenzie et al. [1989] and Mandell et al. [1989], however, found at bone scintigraphy in MED a unilateral avascular necrosis (AVN) superimposed on MED. Koppers [1982] stated that increased uptake of the isotope in MED was a sensitive indicator of activity of the disease.
Magnetic Resonance Imaging (MRI) data on MED are still scarce and are summarized in chapter 5.

1.5.5 Genetics

Fairbank [1947] suggested that MED was, in most cases, not inherited. Later, familial occurence without giving a specific mode of inheritance was reported [Waugh 1952]. A genetic form of multiple epiphyseal disturbances was documented in 1944 [Hermodsson 1944], and later [Christensen et al. 1955, Shephard 1956, Elsbach 1959]. Jackson et al. [1954] and Maudsley [1955] suggested a 'Mendelian unifactorial dominant gene', with high penetrance. This autosomal dominant type of inheritance might be differentiated for a gene for the mild and for the severe form of the disease, as an example of genetic heterogeneity [Barrie et al. 1958]. Jacobs [1968] claimed a 'different locus on the same gene', accounting for differently affected joints of a particular family. Hoefnagel et al. [1967] reported on a family and concluded that there was 'autosomal dominance with a high degree of penetrance'. Incomplete penetrance or variation of expression of the gene is also possible. Mena and Pearson [1976] described a family with 'autosomal dominant inheritance of the second generation, whereas the third generation did not show dominant inheritance'. Juberg [1977] disagreed with this statement.
Juberg and Holt [1968] and Gamboa and Lisker [1974] suggested a recessive form of multiple epiphyseal dysplasia. Bercu [1971] stated that the disease has both dominant and recessive characteristics. Finally, isolated cases have been described [Freiberger 1958, Kozlowski and Bartkowiak 1965, Munk and Boldo 1961], suggesting the occurrence of de novo mutations.
To conclude, autosomal dominant inheritance is the main form of inheritance, although autosomal recessive inheritance and spontaneous mutations have been described in a few publications.

1.5.6 Pathology

MED was originally defined as a 'developmental error resulting from some unknown cause' [Fairbank 1947]. Bone morphology showed irregularity of the epiphyseal plates, pronounced disorganisation of cartilage cell columns, with clustering of cartilage cells in dispersed and irregular heaps [Anderson et al. 1962]. The abundant intercellular cartilage matrix was abnormal with cleft formations and areas of degeneration, and cartilage tongues persisting in the metaphysis. Manganese-deficiency in rats produced a similar localized epiphyseal dysplasia in their offspring [Hurley and Asling 1963].
Femoral growth plate cartilage was analysed from a possible MED case with femoral osteosarcoma [Hunt et al. 1967]. This patient had MED of the hips, the knee and possibly the spine; he also developed a slipped capital femoral epiphysis of the right hip. However, the spinal and metaphyseal involvement make the diagnosis of MED less certain, as indicated in some text books [Tachdjian 1990, Resnick 1995].
An unclassified observation is a MED patient (62 years old) with osteoporosis and an unusual isoenzyme pattern of serum alkaline phosphatase, caused by complex formation between serum alkaline phosphatase and immunoglobulin G of the lambda class [Nagamine and Ohkuma 1975]. Also, possible MED in beagle dogs [Rasmussen and Reimann 1973, Rasmussen 1975] cannot serve as a proper model for human

MED, because the animal form resembles chondrodysplasia punctata, as has been mentioned by others [Spranger 1976]. A three generation family with primary idiopathic osteoarthritis [Katzenstein et al. 1990] does not meet the criteria for MED. Electron microscopy studies in MED [Stanescu 1975, Stanescu et al. 1993] demonstrated ovoid to rounded chondrocytes, largely occupying the greater part of the lacunae. The chondrocytes contained many large vacuoles, with a delicate, single limiting membrane. Some vacuoles coalesce into one another, giving a mesh-like appearance. The vacuoles had an electron lucent background material in which electron opaque granules and flakes and delicate filaments are present. These abnormalities could suggest a storage disorder involving the chondrocytes [Stanescu 1975, Stanescu et al. 1993].

1.5.7 Management

There is no therapy for MED. The treatment is symptomatic [Apley and Solomon 1993]. In four children with MED, gait disturbances, loss of range of motion and contractures due to synovitis were responsive to physical therapy, splinting and non-steroidal anti-inflammatory drugs [Patrone and Kredich 1985]. Spranger [1976] suggested that active arthritis is not a part of MED, and its presence suggests a different diagnosis. Remodeling may be stimulated by continuous passive motion of major joints, especially hip and knee [Tachdjian 1990]. In severe joint incongruity or axial deformities, corrective osteotomies, such as pelvic and distal femoral osteotomy, may be indicated. Osteotomies performed before closure of the epiphyseal plates gave only palliative correction and deformities tended to recur [Amir et al. 1985]. However, disabling deformities, also in growing children, are an indication for surgery, also when there are loose bodies in the joints. Degenerative osteoarthritis may be treated by total joint arthroplasty.
Patients may also benefit by counseling about body weight and job choices. Reduction of late osteoarthritic change may be achieved by restriction of physical activities in sports, work and exercise [Murphy et al. 1973]. Primary prevention is only possible by means of genetic counseling [Maroteaux 1979].

1.5.8 Differential Diagnosis

MED is often confused with numerous other osteochondral dysplasias.

Perthes' disease
Differentiation from bilateral Perthes' disease is a common problem [Herring and Hotchkiss 1987]. Misdiagnosis may lead to unhelpful or unnecessary tratment. Among series of Perthes' disease cases, some proved to have MED [Andersen et al. 1988, Griffiths and Witherow 1977, Mau et al. 1960, Monty 1962]. Ikegawa et al. [1991] described a boy with MED, myopia, hearing loss and unilateral Perthes' disease.
Hip radiographs in MED show no avascular necrosis nor fragmentation and regeneration, as is the case in Perthes' disease; the acetabula have loss of definition with a scalloped subchondral bone plate [Tachdjian 1990]. Bone scans in MED show a normal radionuclide uptake

in contrast to uptake in Perthes' disease. Unilateral avascular necrosis superimposed on MED, with lack of radionuclide uptake in MED, was reported [MacKenzie et al. 1989, Mandell et al. 1989]. Perthes' disease is bilateral in 15% of the cases, but it is rarely symmetric [Crossan et al. 1983]. Moreover, Perthes' disease is transient (in contrast to MED), with initial worsening followed by healing. MED shows only steady progression during growth [Crossan et al. 1983]. Radiographs of other joints and family history may help differentiate MED from bilateral Perthes' disease.

Spondyloepiphyseal Dysplasia (SED)
In MED mild spinal deformities may occur, of a lesser degree than in spondyloepiphyseal dysplasia (SED). In SED, the limbs are only moderately involved but the vertebrae show platyspondyly. There are at least two types of SED, spondyloepiphyseal dysplasia congenita, with an autosomal dominant trait, and spondyloepiphyseal dysplasia tarda, an X-linked recessive trait [Tachdjian 1990]. The congenita type shows one variant with severe coxa vara and another with mild coxa vara [Wynne-Davies and Hall 1982]. In both forms, the vertebrae are flattened and the odontoid processus is dysplastic and its ossification delayed. Associated anomalies in SED congenita are cleft palate, myopia, deafness, cataract and talipes equinovarus. SED tarda is characterized by flattened vertebrae and narrowed disc spaces; ossification of the upper and lower anterior margins of the vertebral bodies is absent [Wynne-Davies et al. 1982]. The proximal epiphyses of femora and humeri are minimally involved; distal epiphyses of the long bones and hands and feet are not affected. Analogous to the publications of MED of Wolcott and Rallison [1972], Stösz et al. [1982] described two sibs with SED and diabetes mellitus. MacDermot et al. [1987] described a family with SED congenita with myopia and deafness analogous to Pfeiffer et al. [1973].

Pseudoachondroplasia (PSACH)
Resembling achondroplasia, this disorder is characterized by disproportionately short-limbed dwarfism; however, the head and face are normal and there is widespread epiphyseal involvement. It has more generalized skeletal abnormalities than in MED, like metaphyseal splaying, and pelvic hypoplasia [Apley and Solomon 1993]. Pseudoachondroplasia and MED might be closely related conditions [Stanescu et al. 1993]; Langer et al. [1993] suggested that MED represents the mildest end of the spectrum of achondroplasia. Vertebral anomalies in mild PSACH usually resolve with time; therefore, it may be difficult to distinguish between mild PSACH and MED after puberty [Rimoin et al. 1994].

Dysplasia Epiphysealis Hemimelica (Trevor's disease)
Here one sees asymmetrical abnormal cartilage proliferation and associated enchondral ossification. It affects only half of the epiphysis (medial or lateral) of one or more joints of one limb or a carpal or tarsal bone. However, simultaneous involvement of the upper and lower limb was reported [Cruz-Conde et al. 1984]. Upon adulthood, the lesion manifests itself as irregular bony masses similar to an exostosis, protruding from the epiphysis. The disease is extremely rare, and no hereditary or familial factor has been demonstrated [Tachdjian 1990, Op de Beeck et al. 1993].

Chondrodysplasia Punctata
This is an epiphyseal dysplasia, characterised at birth by short-limbed dwarfism. Synonyms are dysplasia epiphysealis punctata, dysplasia epiphysealis congenita and stippled epiphyses. At birth, the epiphyses show calcifications that may be resorbed during the first year of life. Epiphyses may ossify in an irregular fashion. Chondrodysplasia punctata is a generalised, multisystem disorder producing facial abnormalities, vertebral anomalies, asymmetrical epiphyseal changes and bone shortening. The autosomal dominant form is relatively mild

and is known as the Conradi-Hünermann syndrome; the autosomal recessive form, in which the stippled calcifications occur primarily in the hips and shoulders, bears a poorer prognosis, and is lethal during the first year of life [Resnick 1995]. Herring [1976] described a case of rapidly progressive scoliosis in, as this author named it, MED, which might have been chondrodysplasia punctata. Silverman reported three cases of chondrodysplasia punctata; the end-result resembled MED. He concluded that the two conditions were different stages of the same lesion [Silverman 1961]. This opinion is not generally accepted [Jacobs 1973].

Mucopolysaccharidoses
In some types of mucopolysaccharidosis the epiphyses of the long tubular bones ossify irregularly and they are broad and flattened, e.g. mucopolysaccharidosis IV (Morquio syndrome). However, the clinical features are different and there are distinctive vertebral abnormalities. In patients with MED the urinary mucopolysaccharides are within normal limits in contrast to patients with mucopolysaccharidosis [Hunt et al. 1967].

Cretinism
This leads to progressive and widespread epiphyseal dysplasia, if untreated. Other biochemical and clinical abnormalities and mental retardation will lead to this diagnosis [Apley and Solomon 1993].

Hereditary Progressive Arthro-ophthalmopathy (Stickler's Syndrome)
Epiphyseal changes are similar to those in MED. However, in Stickler's syndrome there are severe progressive myopia (leading to blindness), conductive hearing loss, cleft palate, and minimal platyspondyly. The inheritance is autosomal dominant [Tachdjian 1990]. The disease is caused by mutations in the COL2A1 gene [Vikkula et al. 1994] or the COL11A2 gene [Vikkula et al. 1995].

Still's Disease
Still's disease, described in 1897, is the most aggressive form of juvenile arthritis. Severe systemic symptoms are associated with multiple joint involvement [Lovell and Winter 1986]. The epiphyseal abnormalities, however, may be asymmetric and are associated with effusions and juxta-articular osteoporosis.

Meyer's Dysplasia
This is an epiphyseal dysplasia of the femoral heads, often misdiagnosed as Perthes' disease. The femoral heads in young children show irregular ossifications and flattening of the capital epiphyses. There is no subchondral fracture line, nor an increased density of the epiphysis as is frequently seen in Perthes' disease. There is no risk of developing a diffuse epiphyseal dysplasia [Meyer 1964, Resnick 1995, Khermosh and Wientroub 1991].

Tricho-Rhino-Phalangeal Dysplasia (TRPS)
TRPS is an autosomal dominant inherited disorder. Epiphyses of the middle phalanges are cone shaped, distal phalanges are short and first metacarpals are slightly shorter. Also mild retrognatia, an enlarged nasal tip, a long philtrum and fine, sparse hair demonstrate the generalised nature of the mutation [Beals 1973]. It is (often) associated with a chromosome-8 deletion.

Angel-Shaped Phalango-Epiphyseal Dysplasia (ASPED)
ASPED is an autosomal dominant disorder, with epiphyses of the middle phalanges resembling the angels in Christmas trees. Ossification of epi- and apophyses of the long bones is delayed

in childhood, leading to severe osteoarthritic changes in the hips in adulthood [Giedion et al. 1993].

Lowry-Wood syndrome
This is the phenotype of two brothers with multiple epiphyseal dysplasia, short stature, microcephaly, nystagmus and mental retardation, possibly a new syndrome, autosomal recessive or X-linked recessive [Lowry and Wood 1975]. Nevin [1986] found two similarly affected sibs, without nystagmus, concluding to autosomal recessive inheritance. However, the specificity of this phenotype is questionable: sibs with Lowry-Wood syndrome without mental retardation and nystagmus [Hankenson et al. 1989] and Lowry-Wood syndrome in association with other diseases have also been described [Lowry et al. 1989, Yamamoto et al. 1995].

1.6 Conclusions

Multiple epiphyseal dysplasia is one of the genetic osteochondrodysplasias, normally with autosomal dominant, rarely with autosomal recessive inheritance. It affects both sexes. The disease is seen from early childhood; children present with pain and stiffness of the joints, gait abnormalities and/or delayed growth. Upon examination there are axial deformities of the extremities, short, stubby hands, and restriction of motion of joints. Radiography shows irregularity and fragmentation of epiphyseal ossification centers and centers for carpal and tarsal bones in children. This can lead to genu varum or valgum and coxa vara. Vertebral bodies sometimes show mild aberrations like anterior wedging.
Pathological studies have revealed an irregularity of the epiphyseal line. Chondrocytes are decreased in number and are found in nests or clusters. Areas of matrix between cartilage columns show degenerative changes like fragmentation and loss of matrix substance. The treatment of MED is symptomatic and the differential diagnosis is rather extensive.

2 The Veldhoven Family with Multiple Epiphyseal Dysplasia (MED): a Clinical and Radiographical Survey

2.1 Introduction

Multiple epiphyseal dysplasia (MED) is a rare disorder. Its prevalence is estimated as 11.2 per 1 million based on index patients, and 16.3 per million, if affected relatives are included [Wynne-Davies 1985]. In the Netherlands, one may expect about 168 to 244 patients with MED: every Dutch hospital cares for one or two patients with MED.
At the Sint Joseph Hospital in Veldhoven (the Netherlands), six patients were seen with bilateral joint problems, especially of the knees and the elbows, with a suspected diagnosis of MED. Upon investigation, these cases were found to belong to a single family. It was the starting point for a family study on the phenotypic spectrum of MED. Here, patients and the complete pedigree will be described.

2.2 Index Patients

From 1963, six patients were seen by dr. A.J.G. Nollen at the Department of Orthopaedic Surgery of the Sint Joseph Hospital. Manifesting symptoms concerned knees and elbows. They either had surgery or conservative treatment. The medical history of these six index cases (and their actual status in 1995-1996) is reported here.

Report of cases

Case A (V-18)
Case A, a girl born in 1978, was seen at the age of 10, having pain in the hips. She had a waddling gait and normal symmetric range of motion of her hips. At 12 years, she had persisting complaints of her hips; she had slight valgus of the knees, pedes planovalgi, and a painless functioning of the hip joints. Radiographs of the pelvis showed slight flattening of the proximal femoral epiphyses *(Figure 2.1)*. At 13 years, there was pain and crepitus of both knee joints, with valgus deformity; radiographs showed irregular epiphyses of the distal femora and proximal tibiae. The diagnosis MED was suggested. At 14 years, she had a good range of motion of the hips. At 16 years, she only complains of the left knee joint. Body height is 155 cm ($<P_3$); the range of motion of the elbow, hip and knee joints is good; an X-ray of the hip joints at 18 years was normal *(Figure 2.2)*.

Case B (IV-66)
The mother of case A, born in 1950, had been operated on both knees. She has no actual knee complaints at age 46. Her height is 152 cm. She has a good range of motion of the knees. Flattened condyles of the distal femora are seen on X-rays, but radiographs of the pelvis and cervical, thoracic and lumbar spine are without abnormalities.

Figure 2.1 *Pelvic radiographs of V-18, at the age of 12 years. Anteroposterior view. There is slight flattening and irregularity of the epiphyses of the femoral heads. The acetabula do not show any abnormalities*

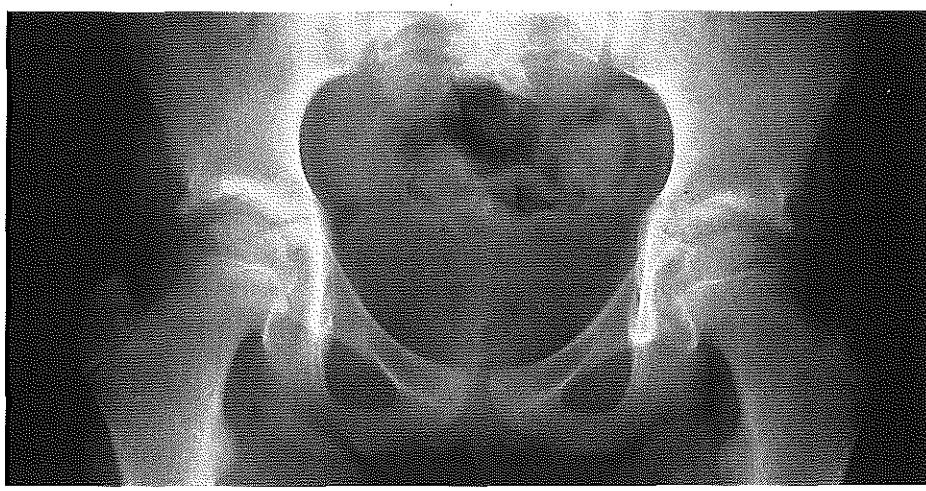
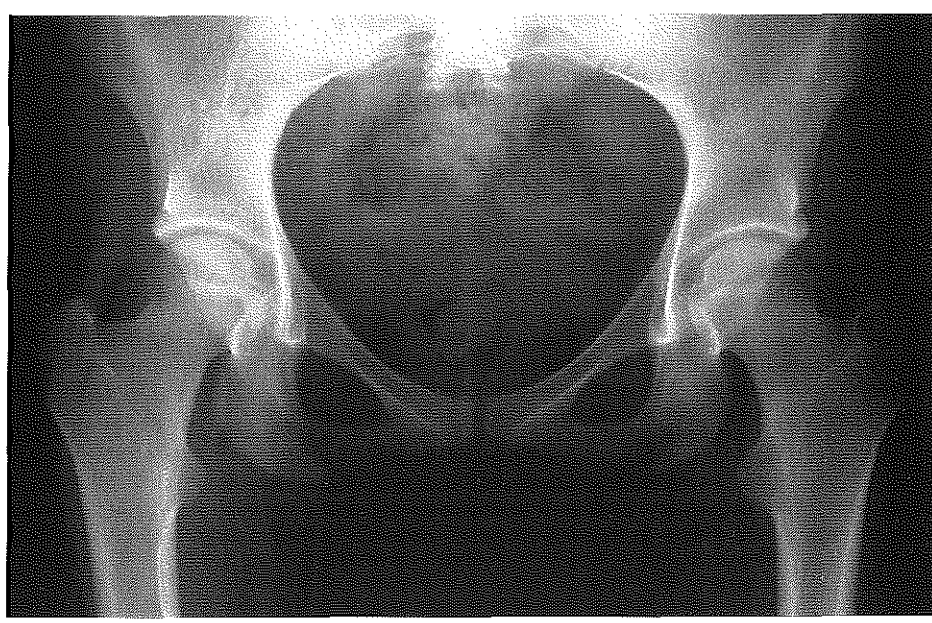


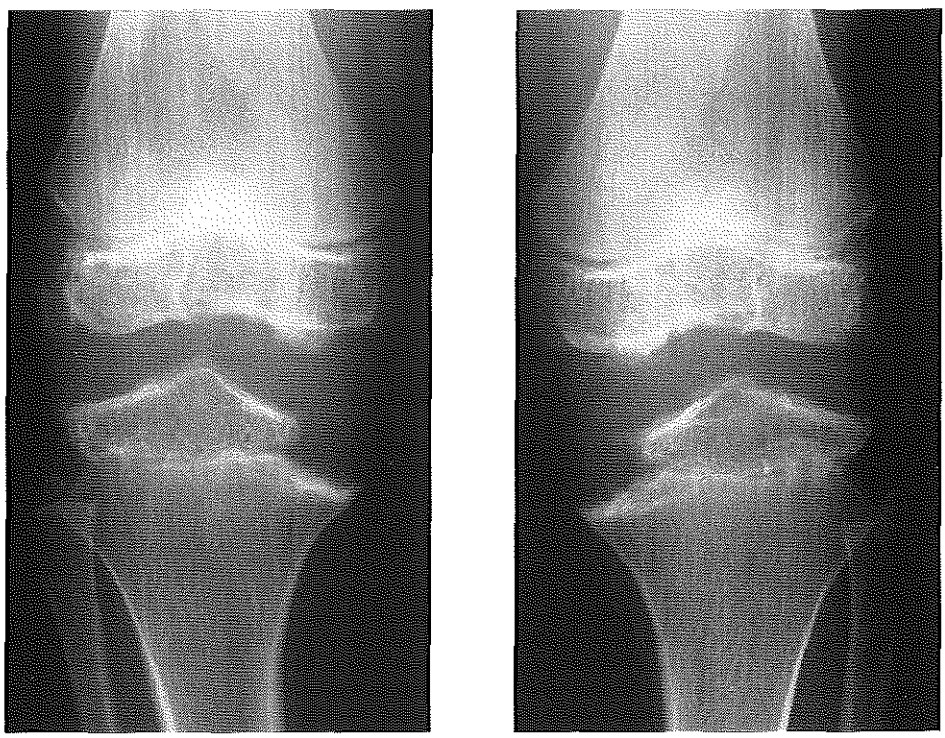
Figure 2.2 *Pelvic radiographs of V-18, at the age of 18 years. The femoral heads have a normal shape*



Case C (IV-2)

Case C, a boy (born in 1976) seen at the age of 6 years with painful legs upon walking, complained of stiff knees. There were slight genua valga, normal range of motion of the knees, short hamstrings and short Achilles tendons. Radiographs of the hip joints were normal. He was treated with stretching exercises for about 3 years. At 12 years, he still had knee complaints: increased genua vara were seen with flattened, irregular epiphyses *(Figure 2.3)*. Also, the epiphyseal ends of the elbows and hands were abnormal. His grandfather and father also had knee and elbow problems. The diagnosis MED was suggested. He had conservative treatment. At age 17, there were no complaints, a 20° varus of both knee joints was found *(Figure 2.4)*, also on radiographs *(Figure 2.5)*. At age 20, there are slight complaints of the knee and ankle joints; his height is 173 cm.

Figure 2.3 *Knee radiographs of case IV-2 at the age of 12 years. The developing epiphyses of the distal femora and proximal tibiae are irregular, resulting in a mild varus shape of the knees*



Case D (III-33)

Case D is a woman, born in 1937. After longstanding complaints of the knees and elbows, she had elbow-surgery at age 26, and bilateral knee surgery at age 26, 29 and 39. At age 52, she was evaluated for locking of the elbows. She experienced varying knee complaints, treated by a physiotherapist. At age 52, she had crepitations of the knees and elbows, with motion limited only in the elbows. The knee and elbow joints showed typical symmetrical flattening of the femoral condyles and osteoarthritic deformations *(Figure 2.6* and *Figure 2.7)*. This prompted the diagnosis of MED. Family history revealed other affected cases (like case C). At age 59, knee and elbow joints are painful. Her height is 156 cm.

Figure 2.4 Cases IV-1 and IV-2, showing the varus knees in case IV-2 (left), whereas the unaffected brother (right) has a normal knee axis

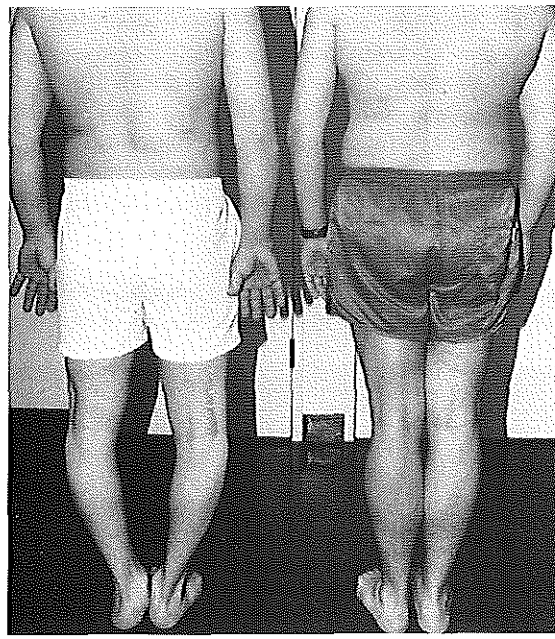


Figure 2.5 Radiographs of the knee joints of case IV-2, at the age of 17 years. Epiphyses of the proximal tibiae are irregular, wedged, and flattended on the medial side, with multiple ossification centers, resulting in a substantial varus deformity

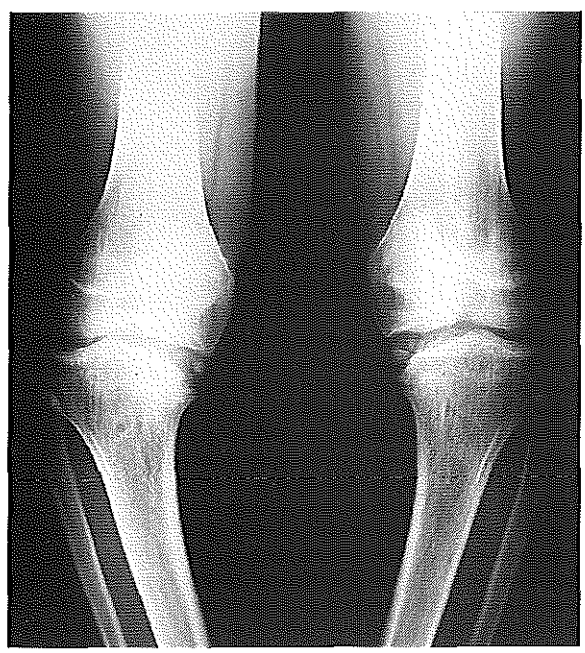


Figure 2.6 Knee radiographs of III-33 (52 years). The distal femoral condyles are slightly flattened and there are signs of osteoarthritis

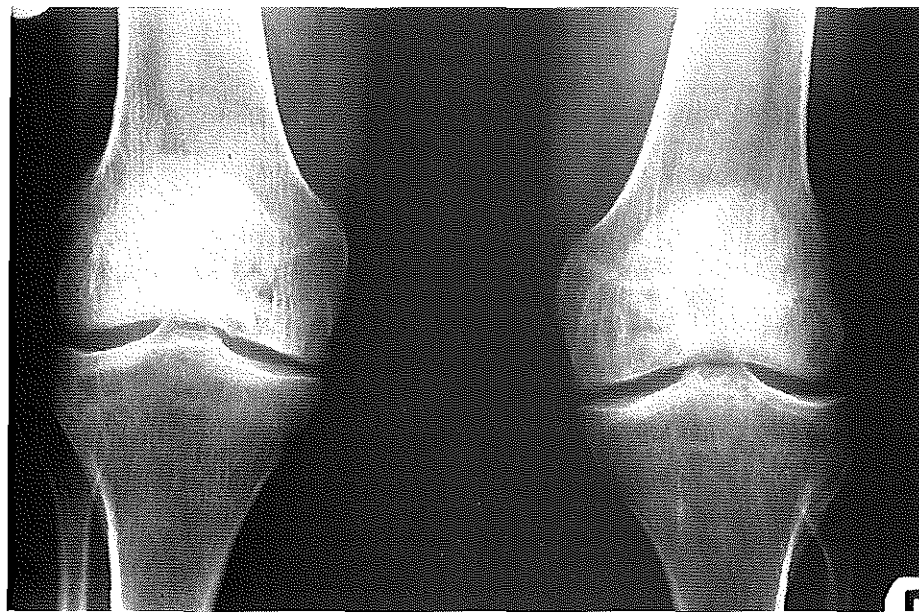
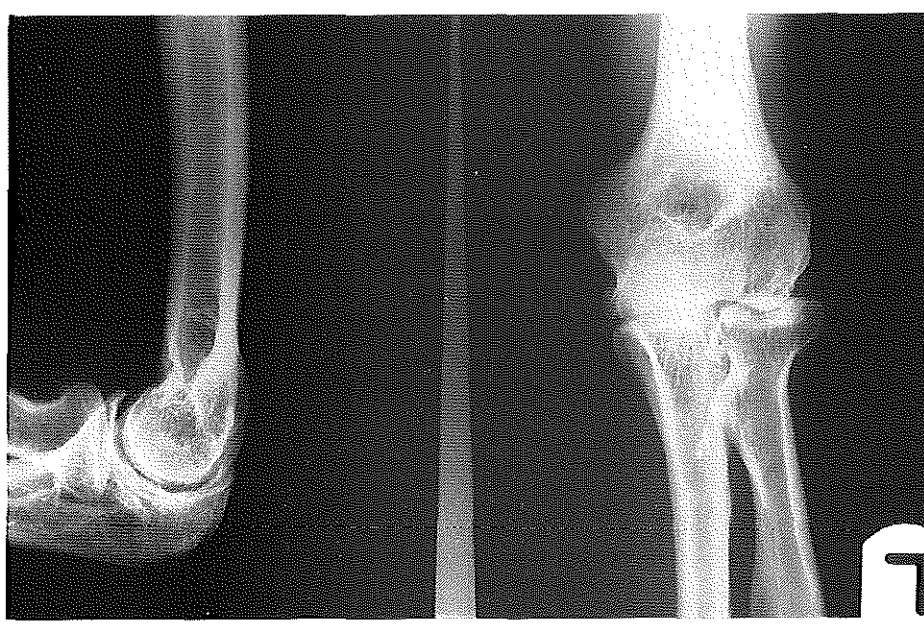


Figure 2.7 Elbow (L) radiographs of III-33 at the age of 52 years, showing osteoarthritic deformations

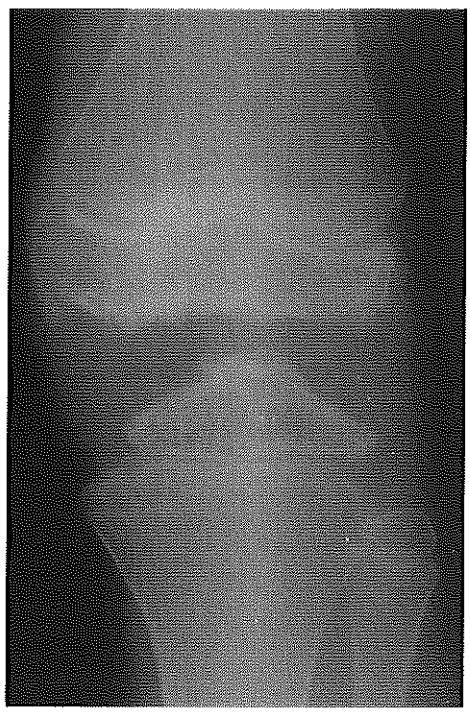


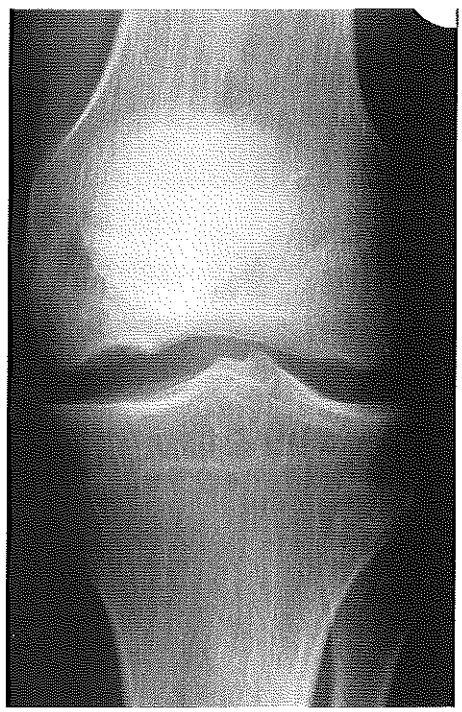
Case E (IV-75)

A boy (born in 1963) was seen at 10 years of age, because of possible 'congenital arthritis of both knees'. At 12 years, there was locking of the left knee, leading to arthrotomy because of osteochondritis dissecans. A radiographic diagnosis 'osteochondritis praecox' was made *(Figure 2.8)*. At the age of 24 years, there were slight knee complaints; there was a normal range of motion and no axial deformities. Flattening of the femoral condyles and an irregular articular surface of the patella was seen *(Figure 2.9)*. The diagnosis MED was made. At 25 years, there are slight complaints of hands, feet, knees and elbows. Height is 184 cm. A younger brother is without complaints.

Figure 2.8 *(Left) Anteroposterior radiograph of the left knee joint of case IV-75 at the age of 12 years. The epiphyses of the distal femur and the proximal tibia are flattened and irregular*

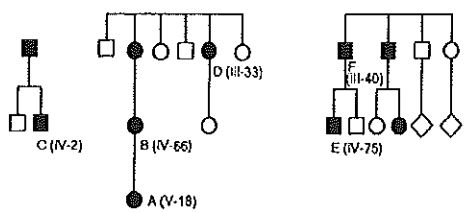
Figure 2.9 *(Right) Anteroposterior radiograph of the left knee joint of case IV-75 at the age of 24 years. The distal femur shows typical flattening, the proximal tibia is normal. The patella shows multiple ossification centers, and is irregular on the lateral view*





Case F (III-40)
Father of case E (age 59 years). He experienced elbow, knee and ankle complaints. Deformation of the distal humeri, proximal ulnae, distal femora and proximal and distal tibiae were observed. His brother and a niece were reported with knee complaints. At that time, cases E and F were not known to be related to cases A-D. Cases E and F were reported as case A2 and A3 before [Versteylen et al. 1988]. At present, case F has complaints of many joints. His body height is 177 cm.
Figure 2.10 shows the pedigrees of these six cases.

Figure 2.10 Pedigrees of the cases A, B, C, D, E and F



■/● : Affected male/female
□/○ : Unaffected male/female
◊ : Sex unspecified

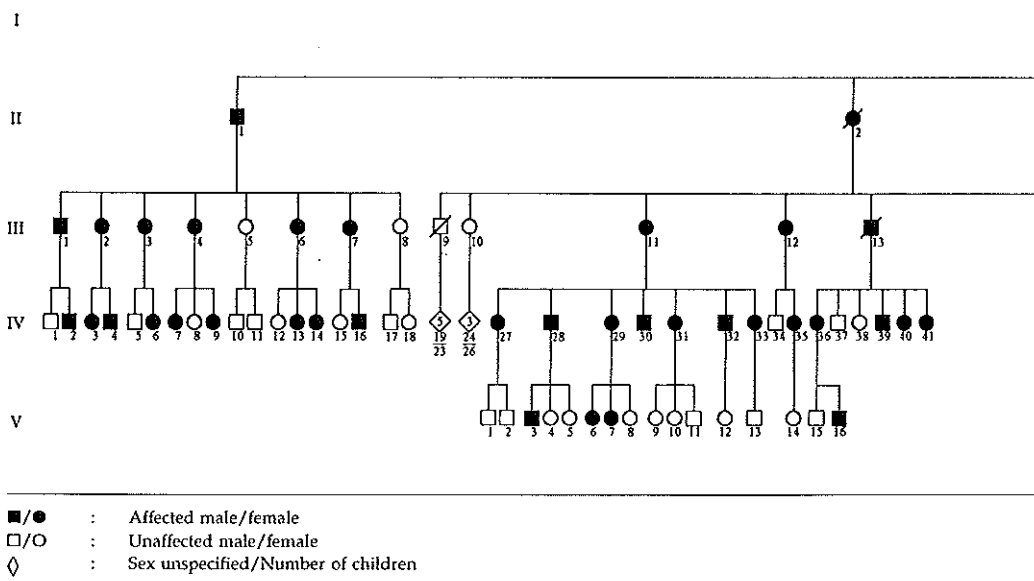
2.3 METHODS

Clinical records and radiographs were reviewed, to confirm the diagnosis MED. This was the starting point for a family study. Through the assistance of the family, we could establish the pedigree of the family, examine individuals (after their full information and free consent) and collect or obtain relevant medical data and radiographs of the joints.

2.4 RESULTS

Cases A, B, C and D *(Figure 2.10)* proved to be related to cases E and F. The complete pedigree consists of five generations and at least 200 individuals. 54 persons have or had MED *(Figure 2.11)*. This was, initially, based on complaints, physical examination and/or radiographical abnormalities. A number of people could not be examined, for instance the three deceased sisters of the first generation (I-1, I-2, I-3). However, I-1 and I-2 were repeatedly remembered as having had knee complaints and difficulties in walking; I-3 was never reported to have had these complaints. The offspring of I-3 is free of joint complaints.
The facial phenotype of the affected persons is normal: however, their height may be short, but proportionate. The gait may be waddling, combined with axial deformities of the legs. The height of the affected individuals may be short to normal. The affected individuals are sometimes shorter than unaffected sibs *(Table 2.1* and *Table 2.2)*. The spine is radiographically normal, meaning there is no hyperlordosis as

Figure 2.11 The complete pedigree of the Veldhoven family with MED, at present



in spondyloepiphyseal dysplasia. The hands and feet may be short and stubby *(Figure 2.12)*.

The joint involvement is striking in this family: knees, elbows *(Figure 2.13)*, ankle, wrist, hand *(Figure 2.14)* and foot joints. No shoulders are involved. Slight deformities of the proximal femoral epiphyses were seen in two childhood cases (IV-16, V-18), but these resolved later on.

Symptoms in affected cases increase during childhood and adolescence, and decrease after puberty. Symptoms returned upon development of osteoarthritis or loose bodies.

The actual pedigree differs slightly from the previous version [Van Mourik et al. 1993]. In the linkage study *(Chapter 3)* two individuals were found to be affected by linkage, and only X-rays gave evidence for this in these two asymptomatic individuals.

Wynne-Davies [personal communication] confirmed the indubitable diagnostic value in MED of the typical radiographical symmetrical abnormalities of the epiphyses of the involved long bones: irregularity of the epiphyses in the younger cases and flattening of the epiphyseal ends and osteoarthritis in the elderly cases *(Figures 2.1–2.9)*.

The details of the individuals involved are listed in *Table 2.1* and *Table 2.2*.

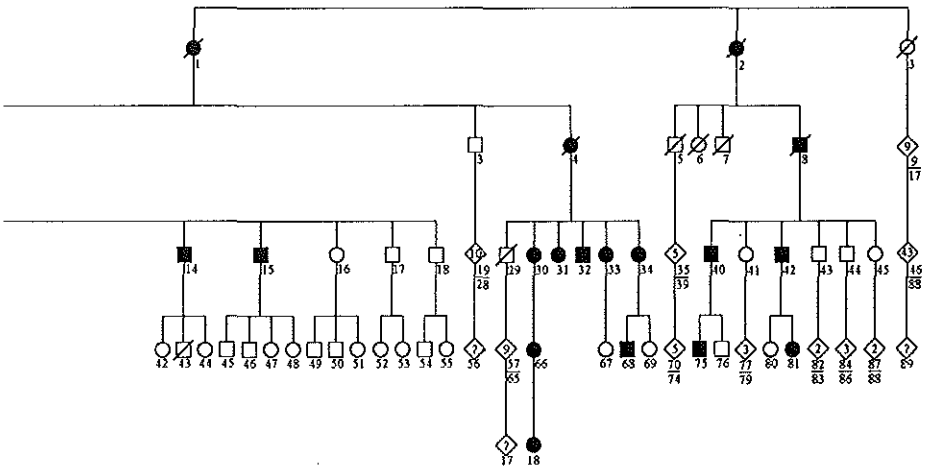

1
2
3
4
5
6
7
8
9
9
17
10
19
28
11
12
13
14
15
16
17
18
29
30
31
32
33
34
35
35
40
41
42
43
44
45
46
88
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
65
66
67
68
69
70
74
75
76
77
79
80
81
82
83
84
86
87
88
89
17
18

Table 2.1 Findings in the Family: Affected Subjects

Case	Sex	Age[1]	Clinical Complaints	Body Height (cm)	Physical Examination	Joint Involvement on Radiographs
II-1	M	86	Knees	?	Varus knees, short hands	Knees
III-1	M	49	Knees	?	Not done	Knees, hands
III-2	F	48	Knees	155 $<P_3$	Varus knees	Knees
III-3	F	47	Knees, elbows, hands	155 $<P_3$	Not done	Knees, elbows
III-4	F	46	Waddling gait, knees	155 $<P_3$	Short hands, crepitation knees, elbows	Not done
III-6	F	42	Elbows, knees	158 $<P_{10}$	Elbow limitations	Knees, elbows
III-7	F	39	Ankles, knees	160 P_{10}	Not done	Knees
III-11	F	68	Knees	146 $<P_3$	Crepitation knees and elbows	Knees, elbow, hand
III-12	F	65	Knees	146 $<P_3$	Joint limitations	Knees
III-13	M	†	Knees	?	Not possible	Knees
III-14	M	62	Unknown	?	Short hands	Knees
III-15	M	59	Knees	?	Not done	Not traceable
III-30	F	75	Knees	144 $<P_3$	Not done	Knees
III-31	F	72	Knees	150 $<P_3$	Not done	Knees, ankles
III-32	M	65	Unknown	165 $<P_3$	Not done	Metacarpals
III-33	F	59	Knees, elbows	156 $<P_3$	Crepitation and OA elbows and knees	Elbows, knees
III-34	F	57	None at present	?	Not done	Knees
III-40	M	59	Knees, ankles, elbows	177 $>P_{10}$	Not done	Knees, ankles, elbows
III-42	M	54	Knees	176 $>P_{10}$	Not done	Knees
IV-2	M	20	Knees	173 $<P_{10}$	Crepitation knees, short hands	Knees, elbows, hands
IV-3	F	18	Knees	160 P_{10}	Joint limitations knees and elbows	Knees, elbow, hand
IV-4	M	17	None	182 P_{50}	Knee crepitation	Knees, elbow, hand
IV-6	F	21	Stiff knees	158 $<P_{10}$	Crepitation knees joint limitation elbows	Knees, elbow, ankles
IV-7	F	17	Ankles, knees	155 $<P_3$	Joint limitations	Knees, elbow
IV-9	F	12	Knees, ankles, elbows	154 $>P_{10}$	Crepitation knees	Knees
IV-13	F	17	Walking stiffness	163 $>P_{10}$	Joint limitation	Knees, elbow, ankles
IV-14	F	16	Locking of knees	165 $>P_{10}$	Crepitation knees	Knees, elbows
IV-16	M	17	Knees, hands, ankles	170 P_3	Joint limitation	Knees, ankles, hands
IV-27	F	43	None	168 P_{50}	Joint limitation	Knees, ankles, hand
IV-28	M	42	Elbows	167 $<P_3$	Not done	Elbows, hands, feet
IV-29	F	41	Stiff ankles and knees	162 $>P_{10}$	Not done	Knees, ankles
IV-30	M	39	Waddling gait	168 $<P_3$	Crepitation knees	Knees, wrist
IV-31	F	37	Knees, ankles, elbows	152 $<P_3$	Joint limitation	Knees, ankles, elbows
IV-32	M	35	Knees	?	Not done	Not done
IV-33	F	30	Stiffness of joints	151 $<P_3$	Not done	Hands, ankles
IV-35	F	40	Knees	161 $>P_{10}$	Not done	Knees
IV-36	F	38	None at present	172 $>P_{50}$	Crepitation knees	Knees, feet
IV-39	M	34	Knees	?	Not done	Knees, elbows, ankles
IV-40	F	33	Knees	?	Not done	Knees
IV-41	F	30	Knees	?	Not done	Knees
IV-66	F	46	None at present	152 $<P_3$	Not done	Knees
IV-68	M	39	Knees, ankles	167 $<P_3$	Joint limitation	Knees
IV-75	M	33	Hands, knees, ankles	184 $>P_{97}$	Joint limitation	Knees, ankles, elbows
IV-81	F	20	Knees, hands	160 P_{10}	Joint limitation	Knees, ankles, hands
V-3	M	07	Knees, elbows	119 $<P_{10}$	Hydrops of knees	Knees, elbows
V-6	F	11	Knees	136 $<P_{10}$*	Not done	Knees, hands
V-7	F	08	Knees, ankles	135 $>P_{50}$	Not done	Knees, hands
V-16	M	07	Stiff knees	132 $>P_{50}$	Slight crepitation of knees	Knees
V-18	F	18	Knees, elbows	155 $<P_3$	Valgus knees and crepitus	Knees, elbows

Age[1] : Age in 1996
† : deceased
? : Height not known
* : On growth hormone replacement therapy

Table 2.2 Findings in the Family: Unaffected Subjects

Case	Sex	Age[1]	Clinical Complaints	Body Height (cm)	Physical Examination	Radiographic Findings
III-16	F	55	None	?	No abnormalities	Knees, ankles, hands normal
III-17	M	53	Unknown	?	Not done	Elbows, hands normal
III-18	M	50	None	?	Not done	Spine normal
IV-1	M	21	None	172 $<P_{10}$	No abnormalities	Knees normal
IV-5	M	23	None	170 P_3	Not done	Knees normal
IV-8	F	16	Elbows	160 P_{10}	No abnormalities	Knees, ankles, elbows normal
IV-12	F	18	Knees	172 $>P_{50}$	No abnormalities	Knees, ankles normal
IV-15	F	20	None	165 $>P_{10}$	Not done	Knees, ankles normal
IV-34	M	38	None	166 $<P_3$	Not done	Not done
IV-51	F	23	Knees	?	No abnormalities	Knees normal
IV-52	F	23	Unknown	?	Not done	Knees normal
IV-53	F	21	Ankles	?	Not done	Ankles normal
IV-67	F	34	None	?	Not done	Knees, wrist normal
IV-69	F	37	None	?	Not done	Not done
IV-76	M	31	None	187 $>P_{50}$	Not done	Not done
IV-80	F	24	None	163 $>P_{10}$	No abnormalities	Knee, ankle, shoulder normal
V-1	M	15	None	178 $>P_{50}$	No abnormalities	Knees normal
V-2	M	12	None	161 $>P_{50}$	No abnormalities	Knees normal
V-4	F	06	None	120 P_{50}	No abnormalities	Knees, elbows normal
V-5	F	05	None	112 $>P_{50}$	No abnormalities	Knees, elbows normal
V-8	F	06	None	118 $>P_{10}$	Not done	Not done

Age[1] : Age in 1996
? : Height not known
P_3 : Third Centile

Figure 2.12 Hands of IV-1 (R) and IV-2 (L). Note the stubby form of the affected hand (left side)

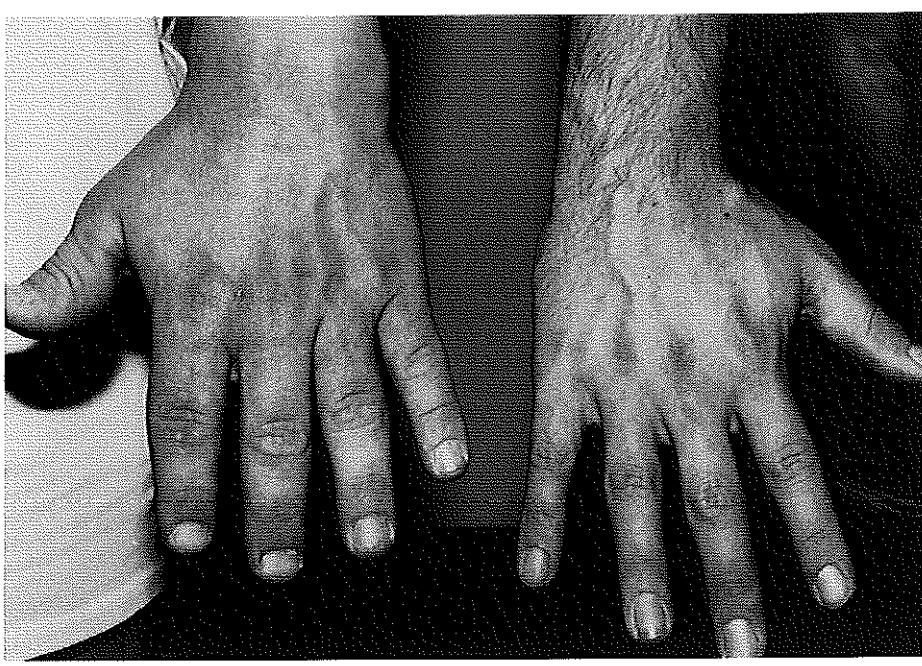


Figure 2.13 Radiographs of the right elbow joint of case V-18 at the age of 10 years, showing irregularity of ossification of the epiphyses of the distal humerus and proximal ulna

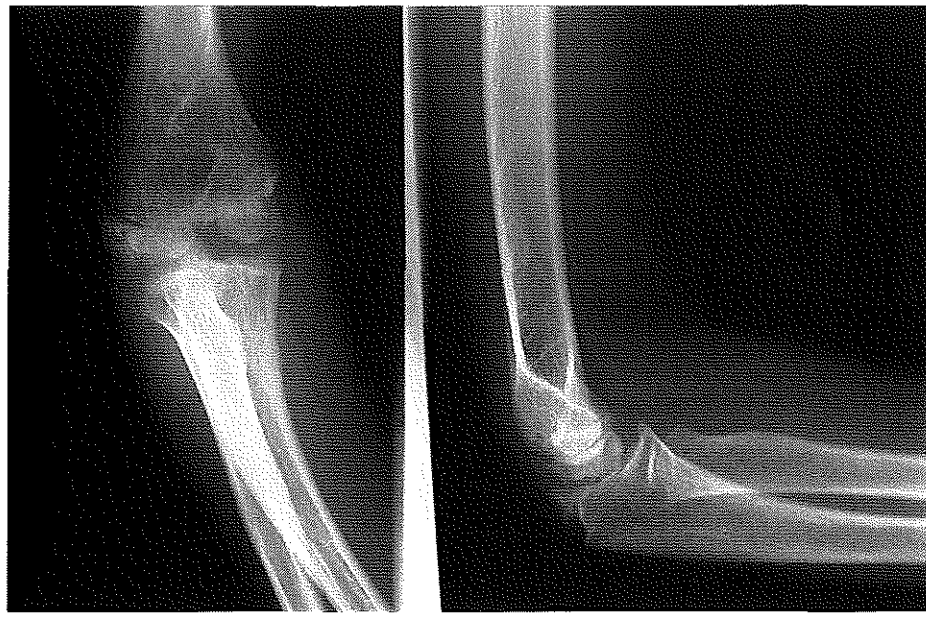
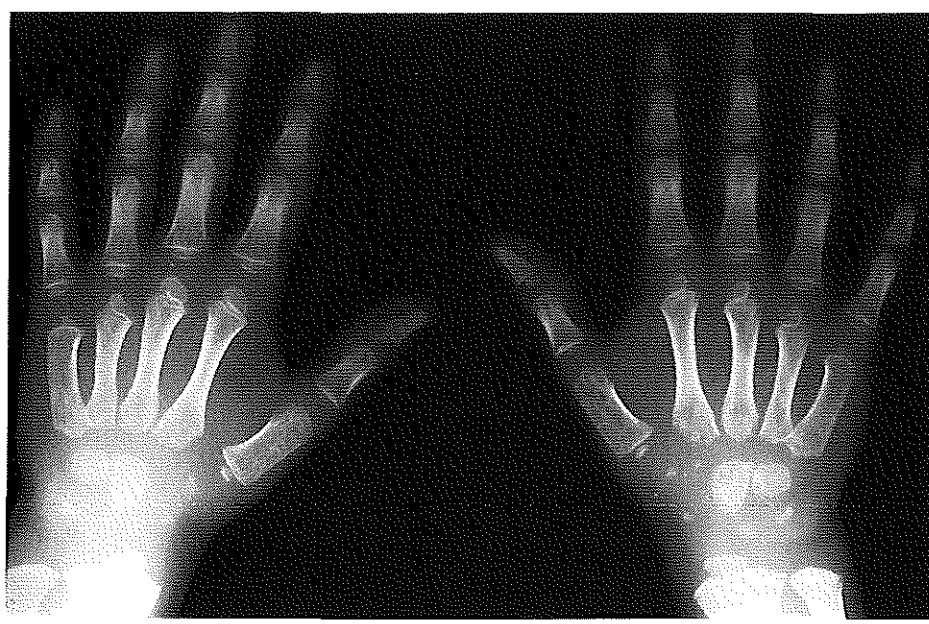


Figure 2.14 Radiographs of the hands of case IV-16 at the age of 9 years, showing irregularity of the ossified epiphyses of the hands and wrists, and the irregularity of the carpal and metacarpal bones



2.5 DISCUSSION

This study reports the largest published family with MED. The other large family had 165 members, with 53 affected individuals [Amir et al. 1985].
Even if there is a general pattern of gradual increase of arthritic symptoms with advancing age, there is clinical variability in the severity of the disorder: sometimes linkage studies may prompt a diagnosis (upon review) in a paucisymptomatic case.
The absence of shoulder involvement and the very rare occurence of hip involvement is notable in this family, contrasting with concurrent shoulder and hip involvement in other families (see *Table 2.3)*, lower extremity involvement [Weinberg et al. 1960], or in hip-only affected families [Diamond 1973]. The latter cases require a differential diagnosis of Meyer's dysplasia. Soodan et al. [1978] described eight cases in two families without involvement of the hip joint; two (asymptomatic) members of these families had knee involvement. Jacobs [1968] also described 8 cases without hip involvement. Concerning the shoulder, Ingram [1991] stated that shoulder involvement was seen in 32% of the involved cases in his series of 50 patients with MED.
Spinal involvement has been described in some cases of MED [Freiberger 1958, Hoefnagel et al. 1967, Lehman and Murray 1981, Murphy et al. 1973, Weaver et al. 1993]. In this family, spinal involvement was not seen, neither radiographically, nor clinically. According to Hulvey and Keats [1969], the frequency of spinal involvement in MED has been shown to vary from 0% in some families to 100% in others. We still have no explanation for the specific involvement of certain joints in this particular family.
Completing data on transmitting relatives (since the 1993 study) we obtained radiographic evidence that there is a very high penetrance in MED; 'clinically' unaffected cases often (this study) show symptomless radiographic features of the disease. In contrast to our earlier report [Van Mourik et al. 1993], we now found evidence that the penetrance of the gene at the radiographic level is complete. A symptomless woman (in 1993) having a son with MED, showed radiographic MED of the knees (case III-25).
As in other dominant disorders, the expression of MED is extremely variable, as regards the number and degree of joints involved. A clinical or molecular explanation for this variation is not yet available; there are no correlations with weight, body height, sex, or profession. MED in this family is relatively benign, rarely requiring operations *(Chapter 6)*.
Height is highly variable. Reduced height, reported as disease specific, of 158 to 188 cm. [Hoefnagel et al. 1967, Weaver et al. 1993] *(Table 2.1* and *Table 2.2)* is not confirmed in this study: unaffected relatives in our study (as in Mena and Pearson [1976]) also may vary extensively in height.
Completion of diagnostic work up by combined clinical and radiographical studies is rather difficult in such a large family, also due to incidental lack of cooperation. Sometimes, diagnostic evidence was obtained from radiographs obtained for diagnosis of MED-unrelated traumatic fractures.

Table 2.3 Number of Cases and Involvement of Joints Described in Literature

Publication	Number of Cases	Shoulder	Elbow	Wrist	Hand	Hip	Knee	Ankle	Foot	Spine
Barrie 1958	15	+	+	+	+	+	+	+	+	?
Berg 1966	4	+	?	–	+	+	+	+	?	–
Bhowmick 1991	1	?	?	+	+	+	+	?	+	?
Brunzlow 1988	2	?	+	–	+	+	+	?	?	?
Christensen 1955	4	+	+	+	+	+	+	+	?	–
Cowan 1963	6	+	?	?	?	+	+	+	?	+
Deere 1995	17	?	?	+	+	+	+	?	?	?
Diamond 1973	23	?	?	?	?	+	?	?	?	?
Emr 1952	2	+	+	+	+	+	–	+	?	–
Fairbank 1947	20	+	+	+	+	+	+	+	+	?
Freiberger 1958	4	+	?	+	+	+	+	+	+	+
Geiser 1954	9	+	?	+	?	+	+	+	?	?
Gibson 1979	10	?	?	?	+	+	+	+	?	–
Hobaek 1961	11	+	+	+	+	+	+	+	+	–
Hodkinson 1962	3	+	?	+	+	+	+	+	+	?
Hoefnagel 1967	12	?	+	+	+	+	+	+	+	+
Hulvey 1969	19	?	?	+	+	+	+	+	?	–
Hunt 1967	2	?	?	?	?	+	+	?	?	+
Jackson 1954	6	?	+	+	+	+	+	+	?	+
Jacobs 1968	8	–	–	+	?	–	+	+	?	–
Juberg 1977	4	?	+	+	?	+	+	?	?	+
Kaufman 1963	2	+	?	+	+	+	+	?	?	–
Koppers 1982	7	?	+	+	+	+	+	?	?	+
Kozlowski 1967	8	+	+	+	+	+	+	+	+	?
Leeds 1960	2	?	?	+	+	+	?	+	?	–
Lehman 1981	24	?	+	–	–	+	+	+	?	+
Levy 1957	1	+	?	+	+	+	+	+	+	–
Lie 1974	13	?	?	?	+	+	+	+	?	+
Litchman 1958	7	+	?	+	+	+	+	+	+	+
Mansoor 1970	9	?	+	+	+	+	+	+	+	–
Maudsley 1955	15	+	+	?	+	+	+	+	?	+
Mena 1976	6	?	+	?	?	+	+	+	+	–
Van Mourik 1993	34	–	+	+	+	–	+	+	+	–
Murphy 1973	24	+	?	?	+	+	+	+	?	+
Ödman 1959	12	–	+	+	+	–	+	+	+	–
Shephard 1956	6	+	+	+	+	+	+	+	+	+
Short 1979	1	+	?	?	+	+	?	?	+	–
Soodan 1978	8	+	+	+	+	–	+	+	+	–
Vizkelety 1964	21	+	?	+	+	+	+	?	+	?
Watt 1952	4	+	+	+	+	+	+	+	+	?
Waugh 1952	3	–	+	?	+	+	+	+	+	?
Weaver 1993	32	+	?	?	+	+	+	+	+	+
Weinberg 1960	45	–	–	–	–	+	+	+	+	?

\+ : affection of the joint by MED has been described
– : the joint has been examined; it was not affected by MED
? : affection of this joint is unknown (neither examined nor described)

2.6 CONCLUSIONS

From this study it may be concluded that:

- A large family with MED in the South-East region of the Netherlands was analysed.
- Elbows, wrists, hands, knees, ankles and feet are involved in this family ; hips and shoulders remain symptom-free.
- Penetrance of the gene is complete; however, the expression is variable.
- The height is inconsistently affected by the mutation.

3 Linkage Studies in Multiple Epiphyseal Dysplasia (MED): Linkage Demonstrated for a Region on Chromosome 1 Containing the COL9A2 Gene

3.1 INTRODUCTION

Literature data *(Chapter 1)* and the extensive family study *(Chapter 2)* confirmed that multiple epiphyseal dysplasia shows autosomal dominant inheritance in the family presented. This allowed finding of the chromosomal localisation of the gene defect in this family. Usually, this location is detected by demonstrating linkage on the same chromosome with a gene earlier identified on the same chromosomal area. Alternative forms of a particular gene are called alleles; if alternative forms exist, the gene is called 'polymorphic'. Any one chromosome bears only one allele at a given locus. When the alleles of two linked genes are separated by crossing over, the new allele combinations formed are called recombinants. The fraction of meiotic events that show a recombination between two loci is called the recombination fraction Θ [Thompson et al. 1991].

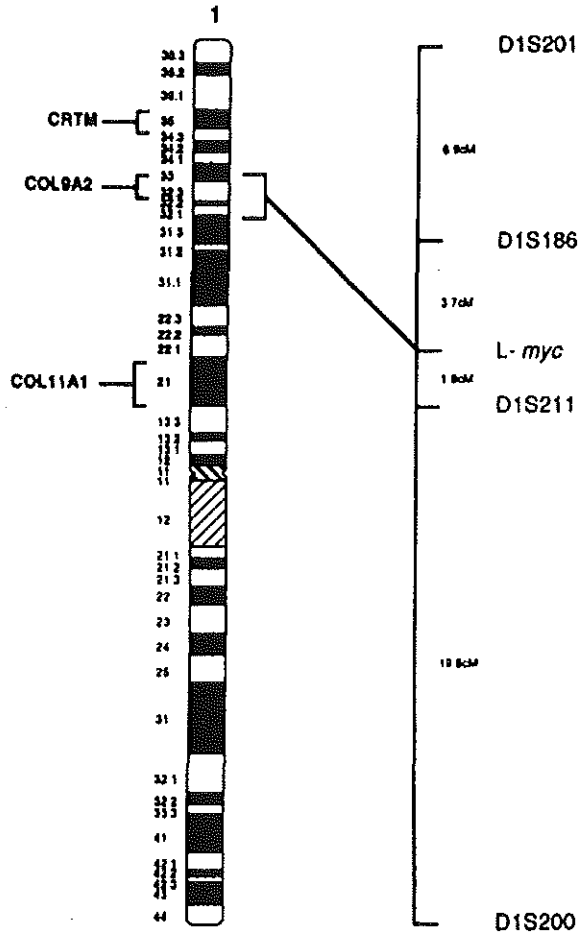
Genetic linkage between two genes is detected by studying pedigrees. Because of the rarity of large pedigrees, indirect methods have been developed. One of the most used methods is the 'log of odds'-method. The chance that the alleles of two loci, f.i. the marker locus and the disease locus, are spread in a pedigree in a given way is calculated under the assumption of no linkage. A similar calculation is performed assuming linkage between the two loci. The ratio between these two values indicates the probability of linkage. In case of more pedigrees these chances are multiplied and the sum of the logarithm of these chances is the lod score (log of odds-score, Z). It is generally accepted that a positive lod score ≥ 3, i.e. chance more than 1000:1, means linkage; a lod score ≤ -2 means no linkage [Pronk et al. 1994].

In multiple epiphyseal dysplasia previous linkage analyses suggested at least three possible loci for MED. The first is EDM1, mapped to a 1.7-centimorgan (cM) region on chromosome 19 [Oehlmann et al. 1994]. Both mild and severe PSACH (pseudo-achondroplasia) have been demonstrated to be located within a 6.6-cM region on chromosome 19, overlapping the MED locus [Briggs et al. 1993, Hecht et al. 1993, Knowlton et al. 1995]. It has been stated that EDM1 and PSACH could be allelic disorders [Stanescu et al. 1993].

Based upon a candidate gene approach, the second locus, EDM2, was mapped to the short arm of chromosome 1 in a region containing the COL9A2 gene [Briggs et al. 1994], which encodes the $\alpha 2$ chain of type IX collagen [Warman et al. 1994] *(Figure 3.1)*. In its vicinity are the polymorphic oncogene L-myc (MYCL) [Nau et al. 1985], and the anonymous DNA markers D1S211 and D1S186. Exclusion of the EDM1 and EDM2 in other families with MED suggests the existence of at least one additional locus [Deere et al. 1995].

It was our objective to determine whether the gene for EDM1 or EDM2 was also responsible for MED in our family. This will be described in this chapter.

Figure 3.1 Ideogram of chromosome 1. On the left side are the locations of the CRTM, COL9A2, and COL11A1 genes. On the right side are the distances between D1S186, L-myc, and D1S211



3.2 Methods and Materials

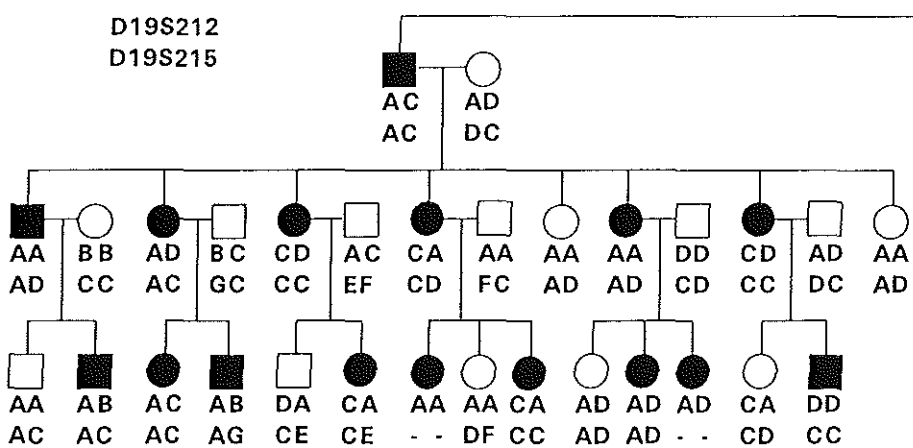
From affected and unaffected members of the family venous blood was sampled, to obtain genomic DNA [Miller et al. 1988]. Family members were classified as unaffected in the absence of a medical history of MED or joint complaints. Genotyping for each of the selected markers was performed by PCR amplification using 50 ng DNA and 30 ng of the appropriate primers (Genome DataBase, Isogen Bioscience, the Netherlands) in 15 µl amplification mixture (10 mM Tris-HCl pH 9.0, 50mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.01% (w/v) gelatin, 200 µM of each dATP, dCTP, dTTP and 2.5 µM dCTP) with 0.06 U SuperTaq DNA polymerase (HT Biotechnology Ltd.). 0.6 µCi α^{32}P-dCTP (10 mCi/ml, 3000 Ci/mmol) was included during the 30 cycles of DNA amplification (1 min at 94°C, 2 min at 55°C and 1 min at 72°C). Sub-

sequently, samples were analyzed on 6.6% denaturating polyacrylamide gels and allelic bands were visualized by overnight exposure of dried gels to Kodak-X-OMAT S film. Linkage analyses were performed using the MLINK and ILINK options of the program package LINKAGE, version 5.10 [Lathrop et al. 1985]. Full penetrance was assumed for the disorder, whereas the disease gene frequency was estimated at 0.0001. The allele frequencies of the genomic markers were obtained from the Genome Database.

3.3 RESULTS

Linkage analysis was performed with microsatellite markers from the EDM1 region (D19S199, D19S212, D19S215, D19S222) and the EDM2 region (D1S186 and MYCL) as determined by Oehlmann et al. [1994] and Briggs et al. [1994]. The results for the EDM1 locus can be seen in *Figure 3.2:* there is no linkage between one of the alleles and the disease. So, the EDM1 locus was excluded.

Figure 3.2 Part of the pedigree and the linkage study with the D19S212 and the D19S215 gene. Recombination between these markers and the disorder is frequently observed



The results for the D1S186 and MYCL gene can be seen in *Figure 3.3:* there is a significant linkage between the used markers and the disorder. The maximum lod score for MYCL proved to be Z_{max}=15.31 at Θ = 0.016 *(Table 3.1).* So, the observed co-segregation of a MYCL-allele is 10^{15} more likely to be due to the close linkage between MYCL and the genetic defect than that it would have segregated in this way by chance.

In the linkage analysis, two patients possessed the at risk MYCL-allele, without clinical complaints. At re-examination they had slight restriction of movement of their elbows and knees. Radiographs showed the typical findings of MED in these two adult patients.

One clinically definite patient with MED did not have the at risk haplotype; this is probably because of a crossover between the L-myc and the genetic defect causing the disease.

Figure 3.3 *Linkage study with the L-myc gene polymorphism for the EDM2-locus. There is complete conformity between the allele called F and the phenotype*

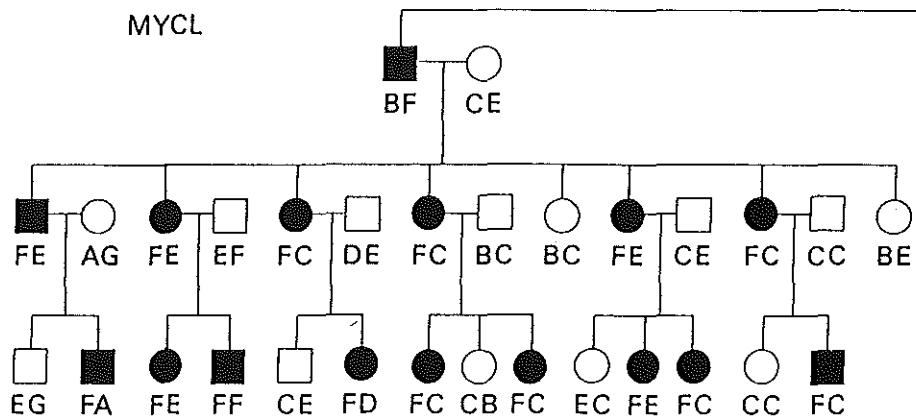


Table 3.1 *Two point LOD scores for MED and chromosome 19 markers (D19S199, D19S212, D19S215 and D19S222) and chromosome 1 markers (D1S186 and MYCL)*

	Recombination Fraction (Θ)				
Locus	0.00	0.05	0.10	0.20	0.30
D19S199	$-\infty$	-8.24	-4.91	-1.96	-0.64
D19S212	$-\infty$	-4.17	-2.30	-0.72	-0.09
D19S215	$-\infty$	-3.97	-2.09	-0.53	0.05
D19S222	$-\infty$	-5.69	-3.46	-1.48	-0.58
D1S186	$-\infty$	15.30	14.09	11.13	7.71
MYCL	$-\infty$	14.83	13.64	10.73	7.32

3.4 DISCUSSION

There are more than 100 disorders of cartilage development, many of which are suspected to be consequences of genetic defects in the macromolecular components of the growing or resting cartilage matrix. Many linkage studies have been performed to detect the involved gene(s) in chondrodysplasias. Most spondyloepiphyseal and spondyloepimetaphyseal dysplasias result from a variety of mutations in the type II collagen locus (COL2A1) on chromosome 12 [Resnick 1995]. EDM2, the type of MED

in this family, proved to be linked to chromosome 1, as published before [Briggs et al. 1994]; their highest lod score was achieved with D1S211 (Z_{max}=5.41 at Θ = 0.04). The short arm of chromosome 1 contains three known genes that encode components of the cartilage extracellular matrix. The COL11A1 gene, which encodes the α1 chain of collagen XI, is located at 1p21 [Henry et al. 1988]; the cartilage matrix protein (CRTM) has been localized to 1p35; and the COL9A2 gene is located at 1p32 [Warman et al. 1994]. COL11A1 and CRTM had been excluded by linkage studies in EDM2 [Briggs et al. 1994, Rimoin et al. 1994]. On the other hand, the COL9A2 gene and the L-myc gene are situated within 200 kb from each other [Briggs et al. 1994]. Since the genetic defect for MED in our family was mapped to the vicinity of L-myc as well, the COL9A2 gene seemed the first candidate gene for the disorder to be screened for mutations. This will be described in the next chapter.

3.5 CONCLUSIONS

From this study it may be concluded that:

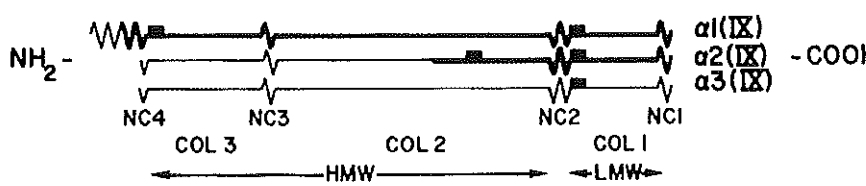
- The locus for MED in this family is at 1p32 with Z_{max}=15.31 for MYCL at Θ=0.016.
- The gene COL9A2, mapped to 1p32, is an obvious candidate for MED.

4 The Mutation in the Gene Encoding the α2 Chain of Collagen IX, Causing Multiple Epiphyseal Dysplasia (MED)

4.1 INTRODUCTION

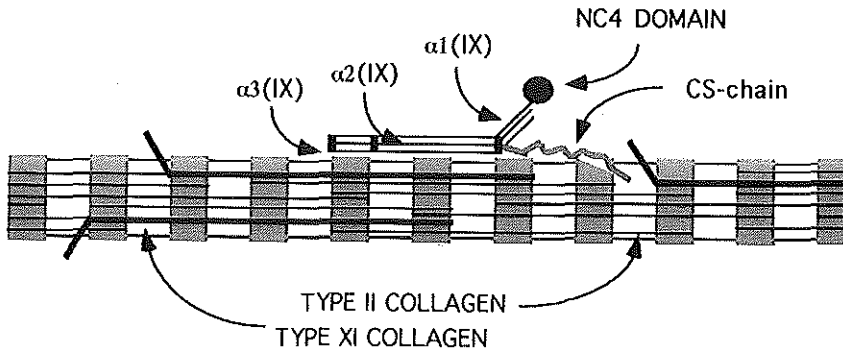
Linkage analysis *(Chapter 3)* localised the genetic mutation of the MED in the family presented in the region of chromosome 1 containing a type IX collagen gene, one of the cartilage specific collagen types; the mutation itself, however, was not yet analysed. Collagen IX is a quantitatively minor collagen belonging to the FACIT (Fibril-Associated Collagens with Interrupted Triple-helices) group of proteins [Olsen 1989, Shaw and Olsen 1991]. It accounts for 1% of the collagenous protein of adult mammalian articular cartilage and 10% in fetal cartilage [Eyre 1991]. It is a heterotrimeric protein, composed of three polypeptide chains, α1(IX), α2(IX), and α3(IX) *(Figure 4.1)*, encoded by the COL9A1, COL9A2 and COL9A3 genes, respectively. The collagen IX molecule consists of three triple-helical domains (COL1-COL3), interrupted and flanked by four non collagenous domains (NC1-NC4) [Mayne and Irwin 1986]. Collagen IX co-polymerizes with collagens II and XI during fibril formation [Mendler et al. 1989] and appears to lie on the fibrillar surface where its large amino-terminal globular domain (NC4) and collagenous (COL3) domain project into the perifibrillar space *(Figure 4.2)* [Bruckner et al. 1988, Eyre et al. 1987, Eyre 1991, Vaughan et al. 1988]. However, Müller-Glauser et al. [1986] stated that type IX is more restricted to the intersection of fibrils. While types II and XI are located in the matrix of both articular and growth plate cartilage, type IX is predominantly pericellular in the calcifying region of articular cartilage and the hypertrophic region of the growth plate [Wardale and Duance 1993].

Figure 4.1 *Molecular form of collagen IX. The molecule contains three collagenous domains, COL1, COL2 and COL3, and four non-collagenous domains, NC1-NC4*



The identification of cartilage components and the delineation of their likely roles has, in the past, relied upon in vitro analyses. Recently, however, molecular genetic analyses of osteochondrodysplasias in mice and humans have provided insight into the in vivo functions of matrix genes expressed in cartilage [Erlebacher et al. 1995, Ala-Kokko et al. 1990, Kingsley et al. 1992]. A wide spectrum of clinical phenotypes

Figure 4.2 Supramolecular structure of collagen IX alongside collagen II. Collagen IX associates via cross-links with the surface of the type II collagen fibrils in an antiparallel fashion. The globular NC4 domain and the triple-helical COL3 domain project out from the fibril surface



is caused by different mutations in the COL2A1 gene [Vikkula et al. 1994, Tilstra and Byers 1994]; autosomal recessive and autosomal dominant osteochondrodysplasias have been shown to be associated with mutations in the genes encoding collagen XI [Vikkula et al. 1995]. Recent experiments have begun to elucidate possible roles of collagen IX in cartilage. Transgenic mice overexpressing a truncated α1 (IX) chain predicted to exert a dominant negative effect on collagen IX function, had a mild chondrodysplasia and progressive osteoarthritis [Nakata et al. 1993]. Mice homozygous for a null mutation in COL9A1 exhibited normal skeletal morphogenesis and growth, but developed progressive osteoarthritis-like changes in articular cartilage [Fässler et al. 1994]. These results strongly suggest that collagen IX may be essential for the maintenance of physical integrity and homeostasis of articular cartilage, rather than for its morphogenesis. Consequently, the collagen IX gene mutations became considered as excellent candidates for inherited late onset mild chondrodysplasia and/or osteoarthritis in humans [Warman et al. 1993, Warman et al. 1994].
The molecular genetic study will be described in this chapter.

4.2 MATERIALS

RNA was extracted by the acid guanidinium thiocyanate/phenol/chloroform method from Epstein-Barr virus (EBV) transformed lymphoblasts of affected and unaffected family members and from cultured chondrocytes of one affected individual [Chomczynski et al. 1987]. First strand complementary DNAs (cDNAs) were synthesized with oligo(dT) primers using the Superscript Preamplification System (GIBCO BRL). PCR primers were designed to amplify the approximately 2 kb cDNA in four overlapping fragments. Nested primers were used for the second round PCR. For amplification by the first round PCR 35 cycles were performed at 94°C for 0.5 min, 62°C for 1 min, and 72°C for 10 min. The second round PCR was performed under the same conditions as the first round PCR except that the annealing temperature was 58°C. The sets of primers used were as follows: 9A2-5 (sense), 5′ CTCCAGGTGGTAGTGCTCGCT 3′ and 9A2-2 (antisense), 5′ GGCTTCCCGCTTGGCACTCAC 3′; 9A2-11 (sense), 5′ GCCACT-

GACCAGCACATCGTG 3′ and 9A2-12 (antisense), 5′ TCAAGGCCCCTTGTAGGATCC 3′; 9A2-7 (sense) 5′ CTGGCGCAGATTAGAGGTCCA 3′ and 9A2-9 (antisense), 5′ ATGCCCCTTC-ACTCCCTGCAG 3′; 9A2-1 (sense), 5′ GCGGATTTCCTGTGTCCAACC 3′ and 9A2-6 (antisense) 5′ AGAGAATCCAGGAAGGCCCTG 3′; 9A2-10 (sense), 5′ CACCAGGGCCTAGCGGGTGT 3′ and 9A2-4 (antisense), 5′ GGCGACCTCTGCCAGTTGCTC 3′. The primers pairs 5/2 and 11/12 were used for first round PCR. The product obtained with the pair 11/12 was analyzed further by using the primer pairs 7/9, 1/6, and 10/4 for second round PCR. All PCR reactions were done in a total volume of 50 µl containing 1xPCR buffer, 200 µM dNTP, 0.5 µM (each) primer and 1U of Taq polymerase. The PCR products were analyzed on 2% agarose gels. All products from affected and unaffected individuals were identical, except for the product obtained with primer pair 7/9. This product migrated as a single band from unaffected individuals and as a double band from affected individuals. Dideoxy-nucleotide cycle sequencing (ampliCycle™, Perkin Elmer), showed a 36 nt deletion in the lower band. To better analyze this deletion, an additional antisense primer 9A2-13, 5′ CAATCCCGGGCTTCCCGTCTG 3′, closer than primer 9 to primer 7, was made and used for second round PCR with the 9A2-7 primer. PCR condition was 35 cycles at 94°C for 0.5 min, 58°C for 1 min, 72°C for 2 min and an additional 10 min at 72°C at the end of the cycles; 0.5 µl of [α-^{33}P] dCTP (10 mM, 2000 Ci/mmol) was added to the reaction. The product obtained with primers 7/13 was analyzed on a 5% sequencing gel and subjected to cycle sequencing as well. For amplifying genomic DNA, two additional sense primers 9A2-14, 5′ CCTGGATCCGACGGCATCGAC 3′ and 9A2-16, 5′ CAATGGGCCCCCTGG-AAAAGC 3′ were synthesized and the primer pairs 14/13 and 16/13 were used for PCR. The conditions were 35 cycles of 95°C for 0.5 min, 64°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 10 min. These amplification products were also cycle sequenced. The products obtained with primers 16/13 from genomic DNA of 79 members of the family were digested with HphI and analyzed on 4% agarose gels. The primers were synthesized on the basis of both published [Perälä et al. 1993] and unpublished sequences. The original GenBank/EMBL file (accession number M95610) has been updated to include the additional unpublished sequences.

4.3 Results

To look for the causative mutation, we started with the reverse transcription-polymerase chain reaction (RT-PCR) on total RNA from EBV-transformed lymphoblasts and from short term cultured chondrocytes of one patient, after admission obtained during arthroscopic surgery. Blood samples from unaffected relatives were used as controls. Nested PCR reactions were used to amplify overlapping cDNA fragments encoding the NC2, COL2, NC3 and COL3 domains *(Figure 4.1)* and the carboxyl half of the signal peptide of the α2(IX) collagen chain. cDNA fragments encoding the NC1 and COL1 domains were amplified without nesting. Analysis of the PCR products on 2% agarose gels revealed that one RT-PCR product, obtained with primers 7/9, containing coding sequences for the COL3 domain migrated as a single fragment in the control individual, whereas the affected individual had two fragments of equal intensity. One fragment was appropriately sized, whereas the second was smaller. The abnormally sized fragment was eluted from the gel and subjected to cycle

sequence analysis. This revealed an in-frame deletion of 36 nucleotides when compared to the wild type sequence *(Figure 4.3)*. PCR with more closely spaced primers demonstrated that the deletion was located close to the amino terminus of the COL3 domain *(Figure 4.4)*.

Figure 4.3

a *The nucleotide sequence of the 3′ region of exon 3 (upper case letters) and the 5′ end of intron 3 (lower case letters). The 5′ splice sequence gt is gc in the mutant.*

b *Exons 2, 3 and 4 splice patterns in wild-type and mutant COL9A2.*

c *The result of cycle sequencing of genomic DNA (antisense strand) from an unaffected individual (lanes 1) and an affected individual (lanes 2). The arrow indicates the point where the affected individual is heterozygous for A and G.*

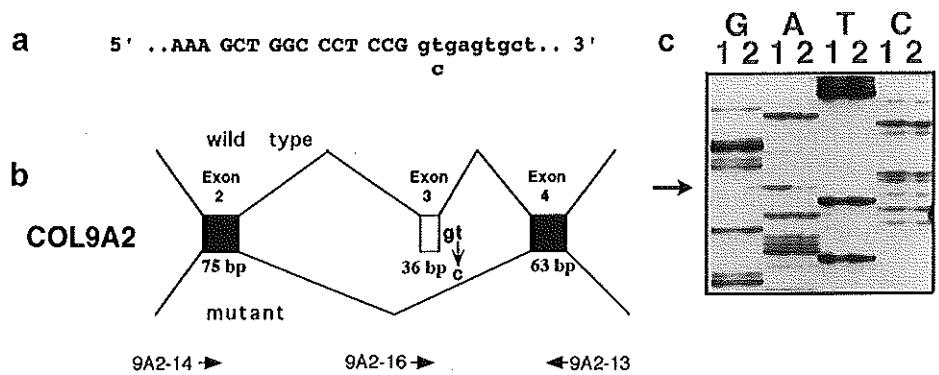
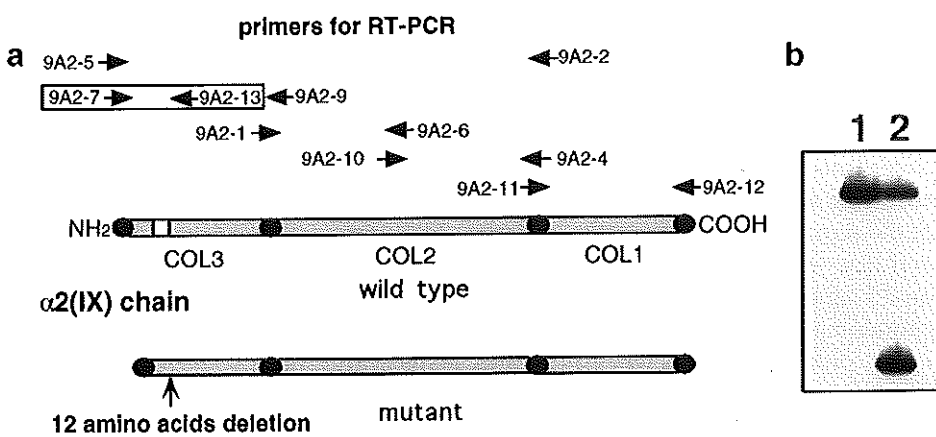


Figure 4.4

a *Diagram showing the location of the 12 amino acid residue deletion in the COL3 domain of the α2(IX) collagen chain caused by the mutation. Within the wild type and mutant α2(IX) chain the triple-helical domains (COL1, COL2, and COL3) are indicated by rectangular areas; the non-triple-helical domains are indicated by the solid spheres. The 12 amino acid residue region in the COL3 domain deleted in the mutant is indicated by an open square.*

b *RT-PCR amplification of α2(IX) mRNA and acrylamide gel electrophoresis with RNA from an unaffected individual (lane 1) and an affected individual (lane 2).*



Since the sequence of the 36 nucleotide deletion corresponded to a single exon encoding the amino terminal region of the COL3 domain of the α2(IX) collagen polypeptide, genomic DNA from within COL9A2 was PCR amplified for further analysis. To amplify this portion of the genomic DNA, PCR primers were synthesized according to the sequences of exons flanking the deleted sequence. The sizes of the genomic PCR products were identical in both affected and unaffected individuals suggesting that the deletion detected at the level of the cDNA was not caused by a deletion of genomic DNA. Cycle sequencing of the genomic PCR products revealed that the sequence of the 5' splice site of intron 3, GTGAG, was converted to GCGAG in one allele of the affected patient *(Figure 4.3)*. This change resulted in the loss of an HphI site in the genomic sequence of the affected allele; loss of this restriction site was used to test for the co-segregation of this change T→C within the family. All affected individuals, including the one who was recombinant for MYCL, were heterozygous for this change. The lod score for this mutation was Z=17.55 at recombination fraction θ=0.0.

4.4 DISCUSSION

In type I collagen dozens of mutations have been identified, most of which represent substitutions of one of the glycine residues by another amino acid, resulting in defective collagen molecules [Kuivaniemi et al. 1991]. In the case of type II collagen, about half of the mutations identified are substitutions leading to the replacement of one of the glycine residues along the polypeptide by another amino acid. This results in at least five different phenotypes; the Wagner syndrome, SEMD, SED, hypochondrogenesis and achondrogenesis. A premature stop codon in COL2A1, resulting in a shortened polypeptide, causes another type of chondrodysplasia, the Stickler syndrome [Knowlton et al. 1989, Vikkula et al. 1994].

The exchange of thymine into cytosine in the COL9A2 gene causes skipping of a complete exon during pre-mRNA splicing. This results in a collagen 9A2 chain lacking 12 amino-acids. This deletion is likely to interfere with normal triple helix formation of collagen IX. Whether the phenotypic effect of this mutation is due to degradation of abnormal heterotrimers causing a deficiency of collagen IX, or whether it is a consequence of copolymerization of abnormal collagen IX with normal collagen molecules is not known.

Likewise, the precise role of the FACIT collagen IX in the structure and function of cartilage is not yet known. Our results, however, confirm previous observations from transgenic [Nakata et al. 1993] and targeted 'knock-out' [Fässler et al. 1994] mouse studies that collagen IX does not appear to have a critical role during the process of skeletal element formation. Patients with EDM2 do not lack specific skeletal elements, nor do they have profound disturbances in skeletal element or shape. Inconsistently, mildly short stature and stubby fingers were identified in our kindred, and other modifying factors (epistatic, environmental, or stochastic) may be involved in these traits. Both the 'dominant negative' and α1 (IX) null mice also have normal skeletal structure.

The process of enchondral ossification, concerning bone growth, also appears to be minimally affected by the COL9A2 mutation. As in EDM1 [Oehlmann et al. 1994],

individuals with EDM2 can have mild reductions in height in comparison to their unaffected siblings [Barrie et al. 1958]. This reduction may be due to altered physical integrity of the growth plate rather than to specific alterations in the normal process of chondrocyte proliferation, differentiation, hypertrophy and apoptosis by which enchondral ossification progresses. Analysis of the murine COL9A1 mutations also fails to detect gross defects in enchondral ossification [Nakata et al. 1993]. It would be premature, however, to conclude that collagen IX does not have any significant role in this process since only one COL9A2 mutation has thus far been identified. It, therefore, remains possible that other COL9A2, COL9A1 or COL9A3 mutations will result in more severe phenotypes affecting skeletal growth. Extreme ranges of clinical severity have previously been associated with allelic mutations in the cartilage extracellular matrix genes COL2A1 [Vikkula et al. 1994], COL11A2 [Vikkula et al. 1995] and COMP [Briggs et al. 1995, Hecht et al. 1995]. Different effects of allelic mutations on gene transcription and translation, or on protein synthesis, assembly, secretion, and supramolecular assembly, are likely explanations for the phenotypic variation. In the absence of detailed biochemical and immunohistological data, the effect of the COL9A2 mutation in the genesis of EDM2 can only be hypothesized. The ability to multiply both mutant and wild type alleles suggests that the mutation does not affect mRNA transcription or stability. Comparable mutations causing in-frame deletions in other cartilage collagen genes have had little effect on polypeptide synthesis or collagen trimer assembly. However, they had profound effect on protein secretion and supramolecular assembly. It is likely therefore, that the COL9A2 mutation in our family causes a dominant negative effect either at the level of protein secretion or supramolecular assembly.

The identification of the COL9A2 mutation as the cause of MED supports a role for collagen IX in contributing to the physical integrity of articular cartilage and/or in participating in the process of cartilage matrix homeostasis. Failure of normal joint function is a unifying feature in all forms of osteoarthritis, although the etiologies by which this failure develops and progresses are heterogeneous. Osteoarthritis in EDM2 may be the consequence of altered physical integrity of cartilage, although joint shape abnormality cannot be excluded. That COL9A2 contributes to the structural integrity of cartilage has already been suggested by its covalent cross-links to collagen II [Van der Rest and Mayne 1988, Wu et al. 1992], its location on the surface of collagen II-containing fibrils, and the projection of its amino-terminal non-triple-helical domain into the perifibrillar space where it is likely to interact with other structural matrix elements [Shaw and Olsen 1991, Vaughan et al. 1988]. It is also possible that a principal role of collagen IX may be in the maintenance of cartilage homeostasis. Rather than interacting with structural matrix molecules, the large amino-terminal domain of collagen IX might bind soluble growth factors, tissue inhibitors of matrix metalloproteases, or extracellular matrix proteins that would otherwise be particularly susceptible to degradation by matrix enzymes. Collagen IX might also either directly, or indirectly, interact with chondrocyte membrane receptors to provide the cells with information regarding the physical characteristics of their surrounding matrix.

4.5 CONCLUSIONS

From this study it may be concluded that:

- In the COL9A2 gene of the studied patients with MED thymine at the 5′ splice site of intron 3 is substituted by cytosine.
- This mutation leads to an inframe deletion of 12 amino acids in the collagen IXα2 chain.
- This mutation is found in every member of the studied family with clinical and/or radiological MED, and is absent in unaffected family members.
- This COL9A2 mutation is the underlying cause for MED in this family.

5 Radiographic Diagnosis of Multiple Epiphyseal Dysplasia (MED)

5.1 Introduction

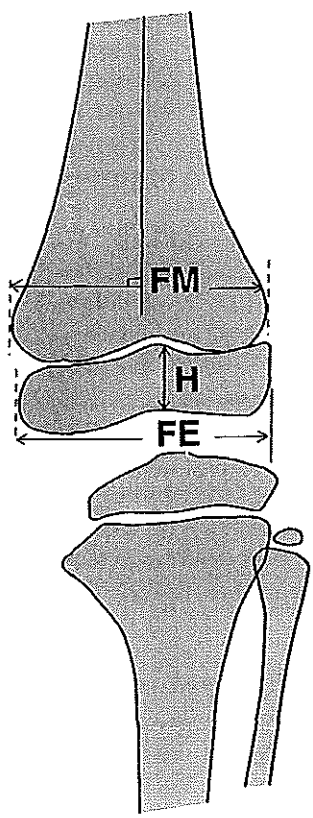
Anthropometry is the science of human measurements, in particular the bones and skull and its segments. Such studies attempted to quantify dysmorphogenesis, and to compare affected members of a family or different cases of a malformation syndrome. Radiography is most often used, but other physical measurements like ratios or proportions, as of span to stature, are used too.
In comparative radiographic anthropometry various methods have been used to measure the bones of the hand skeleton, like angles (such as the carpal angle), relative lengths of various metacarpals (the metacarpal sign) and other bone-to-bone ratios, trying to maximize diagnostic assessment of an easily acccesable skeletal structure. Poznanski et al. [1972, 1984] and Garn et al. [1987] introduced a metacarpophalangeal pattern profile (MCPP) analysis by measuring the length of the tubular bones of the hand and their mutual relationship. Characteristic patterns were described for many inherited syndromes. They suggested that MCPP analysis might be useful in the osteochondrodysplasias. In multiple epiphyseal dysplasia, MCPP analysis was found helpful [Villareal et al. 1992], but uninformative by others [Garn et al. 1987].
The height and width of the distal femoral epiphysis and the width of the distal femoral metaphysis were suggested as an alternative approach [Schlesinger et al. 1986]. The ratio between the height and the width showed a linear correlation. Separate linear regression standards were made for the age groups 6 years or under and those over 6 years of age. There were no differences between the two sexes and acceptably low levels of intra-observer and interobserver variation. This method is much more informative in patients with subtle loss of height of the distal femoral epiphysis, as is seen in MED, than the MCPP analysis [Schlesinger et al. 1986].
Even if linkage or mutation analysis are available in selected MED families, classical reliable imaging technology for diagnostic classification remains the cornerstone of clinical classification. The measurement technology, proposed by Schlesinger, was evaluated in the presented MED family. The results of this study will be presented in the first part of this chapter.

In order to reduce the number of arthroscopies in case of complaints, Magnetic Resonance Imaging (MRI) might be useful. However, only few authors have reported MRI in MED. MRI findings are contradictory in the hip [Toby et al. 1985, MacKenzie et al. 1989, Mandell et al. 1989, Grimm and Just 1992]. MRI of the knee joint in MED is reported in only two cases [Geissler and Ott 1992, Yochum et al. 1995].
Here, we report MRI of the knee joints in six persons with MED, enabling a first evaluation of its additional value over classical radiography.

5.2 Materials and Methods

Radiographs of the knee joints of 15 individuals, younger than 16 years were analysed. There were four children under six years (group 1): two had MED, two were unaffected siblings. In 11 children over six years (group 2) 10 had MED. Genetic linkage was used to assign genetic status. Standard anteroposterior radiographs of the right knee joint were used, because of missing radiographs of the left knee in a number of individuals. Measurements were done as described by Schlesinger et al. [1986] *(Figure 5.1)*. Epiphyseal height (H) was measured from the most proximal point on the distal margin of the epiphysis to the proximal margin of the epihysis in the long axis of the femur. Epiphyseal width (FE) was defined as the greatest width of the distal femoral epiphysis perpendicular to the long axis of the femur. Metaphyseal width (FM) was defined as the greatest width of the metaphysis perpendicular to the long axis of the femur. The epiphyseal height was plotted against metaphyseal and epiphyseal width and compared with the normal standards [Schlesinger et al. 1986].

Figure 5.1 Measurements of the epiphyseal height (H), epiphyseal width (FE) and metaphyseal width (FM). (Redrawn with permission from Schlesinger et al. 1986)



The sensitivity, specificity and positive and negative predictive value of a ratio two or more standard deviations below the mean (≤-2SD), according to Schlesinger et al. [1986], for the diagnosis MED, were calculated. Sensitivity is the percentage of measurements in which the ratio is ≤-2SD in individuals with proven MED; specificity is the percentage of measurements in which the ratio is higher than two standard deviations below the mean (>-2SD) in individuals who are known not to have MED. The positive predictive value defines the likelihood that an individual with a ratio ≤-2SD actually has MED. The negative predictive value is the likelihood that an individual with a ratio >-2SD does not have MED.

The MRI study of the knee joints was performed on six individuals (three boys and three girls) with MED affecting the knee joints. They ranged in age from 7 years to 18 years (mean 12.7 years). In the four individuals without complaints of their knee joints, MRI images were made after informed consent. Two other individuals (cases IV-14 and V-18) had complaints of their knee joints, and were planned for an arthroscopy. In these individuals MRI was performed preoperatively, in order to determine the additional value of MRI in planning an operation.
Plain radiographs in two directions were made of the knee joints. Coronal and sagittal MR images were obtained with a 1,0 T superconducting MRI unit (Philips, Eindhoven). Patients were examined in the supine position. The used pulse sequences were as follows: coronal SE T_1 weighted images (TR 633 msec, TE 16 msec) and sagittal SE T_2 weighted images (TR 2000msec, TE 80 msec); in four patients also a coronal T_1 weighted 3D scan (TR 25 msec, TE 7,4 msec Flip Angle 45°) was made. Sedation was not used.

5.3 Results

Anthropometric measurements are listed in *Table 5.1*. In 11 of 12 individuals with MED, the ratios showed to be ≤-2SD below the mean. In group 1, both affected individuals had a ratio ≤-2SD below the mean, whereas one affected individual over 6 years old had a ratio >–2SD. In one girl radiographs were obtained at 8 and 10 years; they showed identical H versus FM and H versus FE ratios. Likewise, there were no relevant differences between values of the right and left knee. The sensitivity and specificity are high, and the positive predictive value for a ratio ≤-2SD in this family is 100% *(Table 5.2)*. The results are graphically shown in *Figure 5.2*.

MRI imaging of the knee joints of cases V-3, V-6 and V-16 showed the irregular and delayed ossifications of the epiphyses, as documented on radiographs. The cartilage had a normal appearance, without oedema, and neither joint effusion nor loose bodies were seen. Menisci and cruciate ligaments were normal. The ossified parts of the epiphyses had identical morphology on radiography and MRI *(Figure 5.3)*. The epiphysis of case V-3 were stippled on radiography and MRI, slightly resembling chondrodysplasia punctata *(Figure 5.4)*. We concluded that MRI did not provide more information than conventional radiographs about the epiphyseal ends in young individuals with MED.

Table 5.1 Measurements of Epiphyseal Height, Metaphyseal Width, and Epiphyseal Width in 15 Individuals of the Same Family

Case	Sex	Age	MED	Side	H (mm)	FM (mm)	FE (mm)
IV-2	M	13	yes	R	20	77	80
				L	19	76	80
IV-3	F	15	yes	R	20	67	74
				L	20	68	73
IV-8	F	14	no	R	23	77	83
				L	22	75	82
IV-9	F	6	yes	R	12	55	46
				L	12	56	46
IV-13	F	15	yes	R	18	73	80
				L	16	73	80
IV-14	F	12	yes	R	20	75	82
				L	20	75	84
IV-16	M	11	yes	R	13	68	65
				L	14	68	64
IV-75	M	12	yes	R	12	72	76
V-3	M	7	yes	R	15	68	66
V-4	F	4	no	R	18	59	61
				L	18	58	60
V-5	F	3	no	R	16	53	51
				L	16	54	51
V-6	F	8	yes	R	11	51	49
				L	12	54	50
		10	yes	R	13	56	54
				L	12	55	53
V-7	F	5	yes	R	12	54	52
				L	12	53	52
V-16	M	7	yes	R	15	64	59
				L	15	63	60
V-18	F	12	yes	R	16	68	72
				L	15	70	75

Age : Years at radiographic examination
H : Epiphyseal height
FM : Metaphyseal width
FE : Epiphyseal width

Table 5.2 Sensitivity, Specificity and Predictive Values of the H versus FM and H versus FE Ratios in 15 Individuals from the Same Family with MED

	Group 1	Group 2	Both groups
Sensitivity	100% (16-100%)	90% (55-100%)	92% (61-100%)
Specificity	100% (16-100%)	100% (2.5-100%)	100% (29-100%)
Positive Predictive Value	100% (16-100%)	100% (66-100%)	100% (72-100%)
Negative Predictive Value	100% (16-100%)	50% (0.02-100%)	75% (18-100%)

The values in parentheses represent the 95 per cent confidence intervals
H : Epiphyseal height
FM : Metaphyseal width
FE : Epiphyseal width
Group 1 : Age group ≤6 years old
Group 2 : Age group >6 years old

Figure 5.2 *Plots of H versus FM and H versus FE for children ≤6 years old (a and b) and over 6 years (c and d). Solid lines denote the mean, whereas dotted lines represent the limits of two standard deviations*

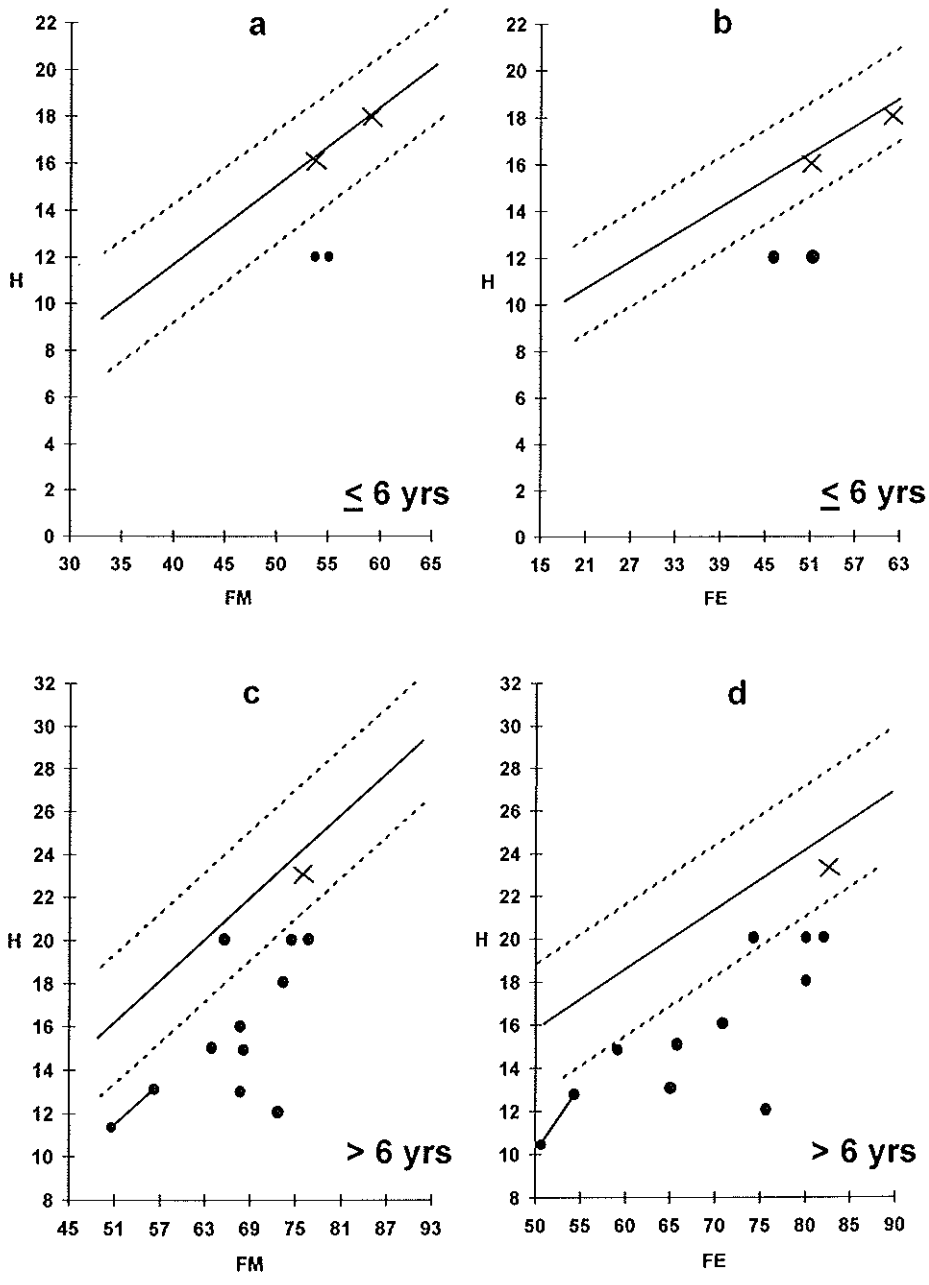


Figure 5.3

a Delayed and irregular ossification of the distal femoral and proximal tibial epiphysis in case V-6 at 11 years (with slight knee complaints)

b Fragmented and irregular ossification centers (MRI, long TR coronal images)

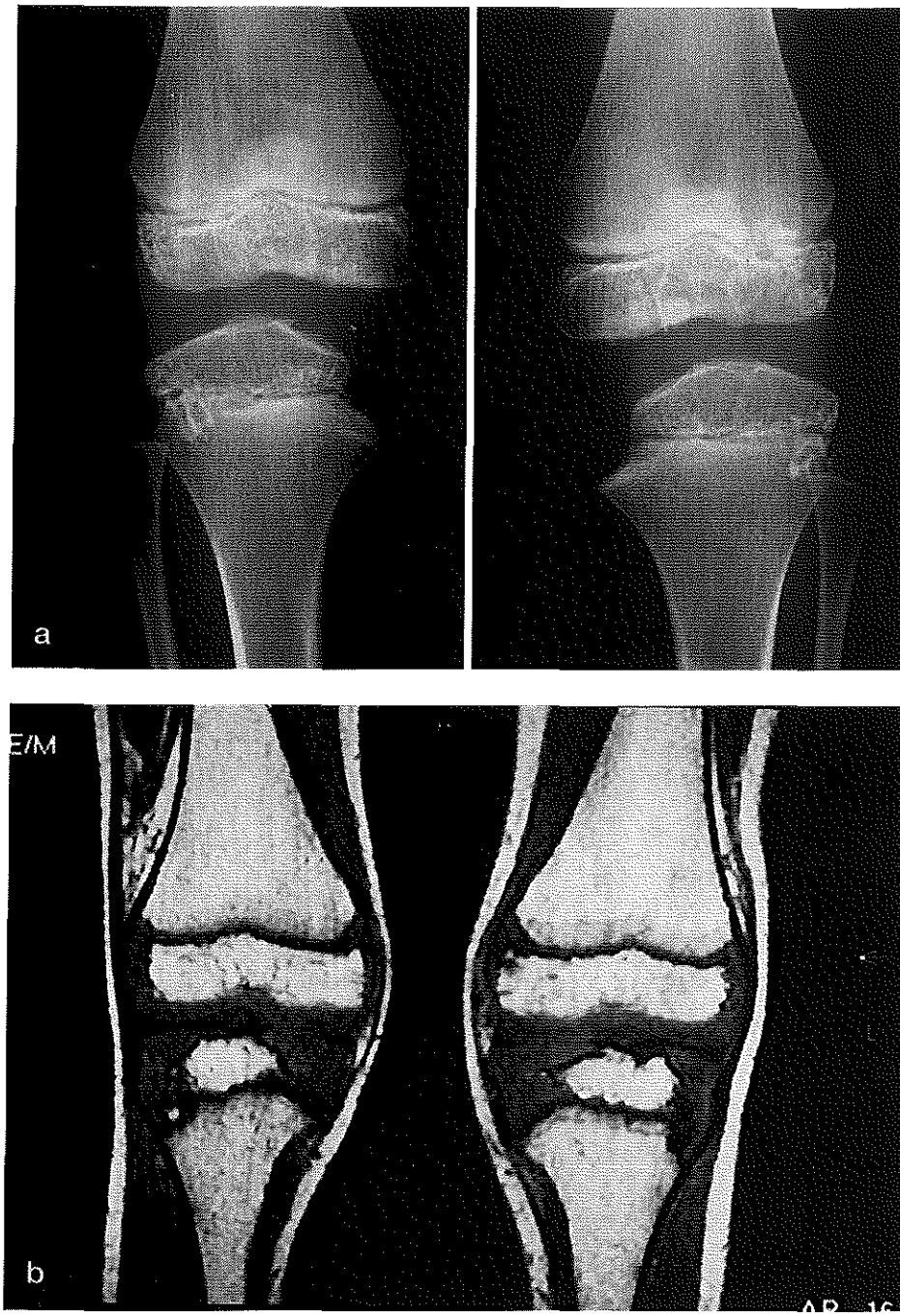


Figure 5.4 Fragmentation of calcified distal femoral epiphysis in case V-3 (age 7 years) (long TR *coronal* MRI *images)*

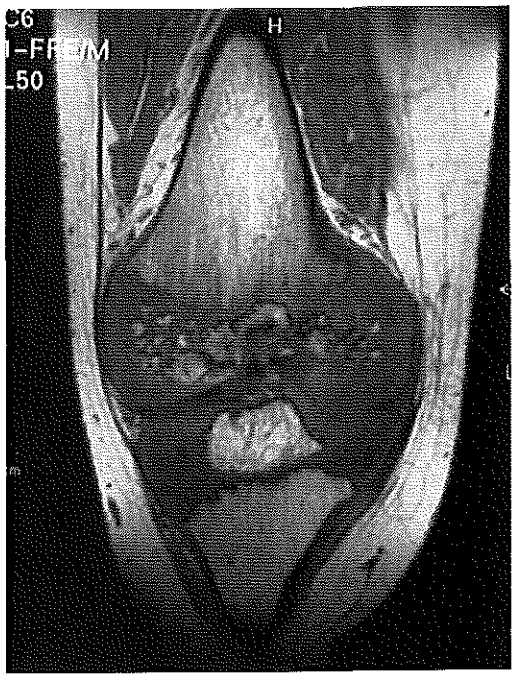
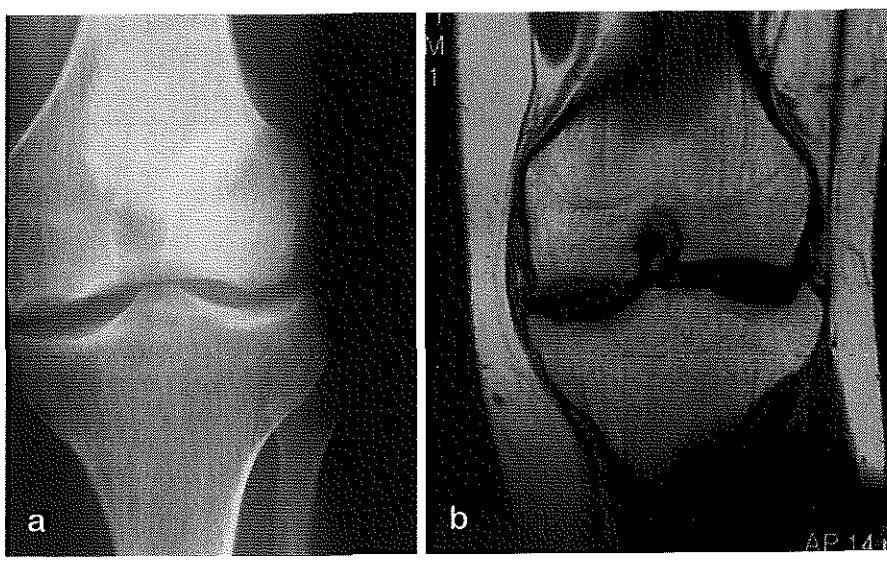


Figure 5.5

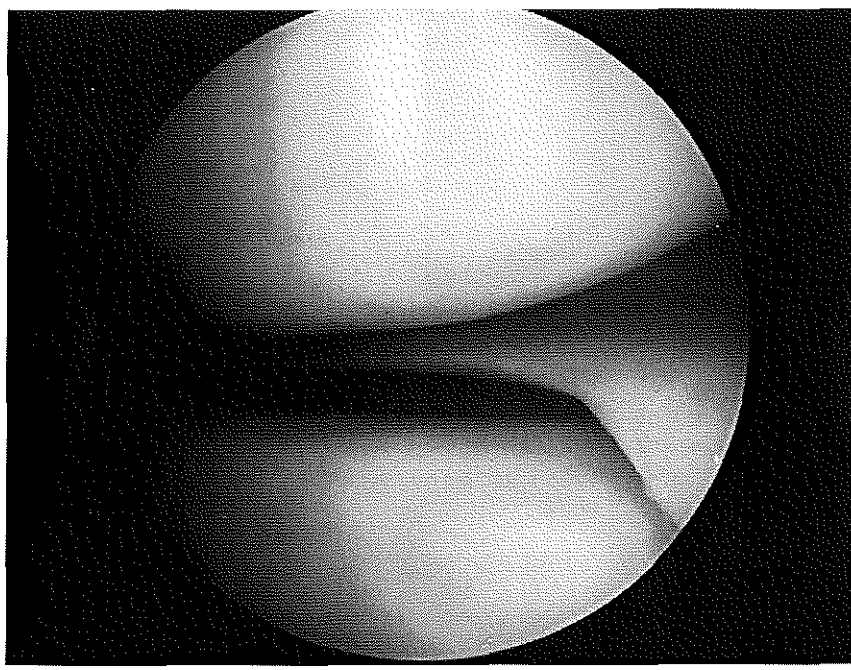
a Typical flattening of the distal femoral condyles in case V-18 (age 18 years) with locking and reduced range of motion of the knee joints (anteroposterior radiograph of the left knee joint)

b Homogeneous bright signal of the bone and the cartilaginous part of the distal femur (long TR *coronal* MRI *images)*



In the older cases (IV-4, IV-14 and V-18) MRI showed a homogeneous bright signal of the former epiphysis on long and short time sequences. The osseous defects, seen on plain radiographs, seemed filled with homogeneous, thick cartilage, without clefts *(Figure 5.5)*. The adolescent cases IV-14 and V-18 had locking of the knee, without free bodies or osteochondritis dissecans on radiography, and normal ligaments and menisci on MRI. So MRI gave no additional information. Arthroscopy was completely normal in IV-14 and V-18: the cartilage had a completely normal appearance, without fibrillation, clefts or osteochondritis dissecans *(Figure 5.6)*. In these MED cases, post-arthroscopy limited range of motion was experienced during a few months, different in early recovery in non-MED cases.

Figure 5.6 Normal medial femoral condyle, proximal tibia, and medial meniscus in case IV-14 (arthroscopic image)



5.4 Discussion

Our better results, compared to the study of Schlesinger et al. [1986], are possibly caused by the fact, that we investigated one large family. Their study revealed that in 54% of the cases with MED the ratio of the epiphyseal height (H) to the epiphyseal (FE) or metaphyseal width (FM) measured ≤-2SD compared to the measurements in healthy children. In our study 92% of the individuals with MED had values of ≤-2SD (2-tailed P-value=0.059). Schlesinger et al. found a higher sensitivity in the age group 1 than in the age group 2; in MED, they did not find a difference between the H versus FM and the H versus FE ratio. We came to the same finding: the two ratios

were comparable. The only case (IV-3) whose value was >-2SD, was relatively old (15 years), making the measurements more difficult because of closure of the epiphyseal plate. In older cases, however, diagnosing MED radiographically is not difficult because of the typical appearance of the epiphyseal ends. Measurements are more useful in early or subtle cases in which the diagnosis MED has not yet been made [Schlesinger et al. 1986]. Usefulness of measurements in early cases was also described by Ingram [1992], who found in 10 (62%) of 16 patients with MED values ≤-2SD when epiphyseal height was compared with metaphyseal width, and in six (38%) of 16 patients with MED values ≤-2SD in epiphyseal height/epiphyseal width ratios.

MRI investigations of MED have seldom been published. Only a single study of the knees and elbows of a 33-year old case showed cartilage hypertrophy on these locations where the epiphyseal bone was narrowed on the normal radiograph. The bone marrow and cartilage seemed normal, there were no free bodies [Geissler and Ott 1992]. Another single case study was on an 11-year MED patient with a double patella [Yochum et al. 1995]. Without a clear description, the published MRI-scan showed normal cartilage and a double patella, and a normal quadriceps tendon. MRI investigations of hip joints of young children revealed irregularity of the proximal femoral epiphyses, a diffusely decreased signal, and a typical garland formation of the epiphyseal plate [Toby et al. 1985, Mackenzie et al. 1989, Mandell et al. 1989, Grimm and Just 1992]. In the present study, no cartilaginous abnormalities of the knee joints were found, nor was cartilaginous hypertrophy seen, as reported by Geissler and Ott [1992]. We suggest that the relatively thick cartilage is epiphyseal cartilage, not replaced by bone, instead of hypertrophic cartilage.
Articular cartilage was also normal on MRI and arthroscopy. Young MED cases have apparently unaffected articular ends of the knees. This is concordant with arthrography of hips in MED [Lachman et al. 1973, 1974, Toby et al. 1985].
The usefulness of MRI is manifold, but no substitute for clinical acumen in internal knee derangements [Dandy 1997]. For lesions of the articular cartilage the sensitivity of MRI is low [Spiers et al 1993]. Similarly in MED, MRI obtains only value for diagnosis when mechanical complaints occur. So, in MED cases with pain without severe physical abnormalities, MRI will be of limited benefit for diagnosis or therapy.

5.5 Conclusions

From this study it may be concluded that:

- Radiographic anthropometry in MED is useful to detect the disease in children and young adolescents.
- In the family studied, radiographic anthropometry has a high predictive value.
- MRI of the knee joint has no additional value in confirming the diagnosis MED.
- Indications for MRI in patients with MED are the same as in regular patients.

6 Pathological Features of Cartilage in Multiple Epiphyseal Dysplasia (MED)

6.1 Introduction

Multiple Epiphyseal Dysplasia (MED) is associated with degenerative changes of affected joints. Orthopaedic interventions are indicated when intra-articular mechanical problems or axial deformities occur [Dahners et al. 1982, Jackson et al. 1954, Kaufman and Coventry 1963, Laurencin et al. 1996, Mansoor 1970, Weinberg et al. 1960]. The intra-articular macroscopical findings were only described by Dahners et al. [1982]. Microscopy showed irregular epiphyseal plates, disorganisation of cartilage cell columns, with clustering of chondrocytes into dispersed and irregular heaps [Anderson et al. 1962]. In electron microscopy the chondrocytes were found to be ovoid or rounded, occupying the greater part of the lacunae [Stanescu 1975, Stanescu et al. 1993].

The presented MED family and its associated collagen IX mutation prompted detailed analysis of cartilage fibrils. Collagen fibrils and hydrated complexes of proteoglycan and hyaluronic acid form the network of cartilage. In fetal hyaline cartilage, these fibrils are heterogeneous in diameter [Bruckner et al. 1988], whereas in adults fibrils are thicker, 30-200 nm. Moreover, collagen fiber diameter not only increases with age, but also with cartilage depth [Lane and Weiss 1975]. The matrix immediately surrounding the chondrocyte, however, has thin fibrils (15-25 nm in diameter) [Poole et al. 1985].
Collagen IX co-polymerizes with collagens II and XI during fibril formation [Mendler et al. 1989] and appears to lie on the collagen-fibrillar surface. It might play a role in the regulation of fibril diameter [Wotton et al. 1988]. Collagen IX accounts for 1% of the collagenous protein of adult mammalian articular cartilage and for 10% in fetal cartilage [Eyre 1991]. In human infantile cartilage, collagen IX was found in all zones of cartilage of rib growth plate. Immunoelectron microscopy showed that antibodies specific for type IX collagen labeled fibrils of all diameters within the entire newborn and infantile growth plate, as did antibodies specific for type II [Keene et al. 1995]. Therefore, it was hypothesized that collagen IX serves as a molecule that binds together the type II collagen meshwork [Smith and Brandt 1992].

Cartilage biopsies, obtained during surgery on six MED cases, were evaluated histologically and electron-microscopically.

Figure 6.1

A Semithin section of MED cartilage (×400)

B Low magnification of chondrocyte of MED cartilage (×7500)

C Cytoplasm of MED chondrocyte with normal looking rough endoplasmic reticulum (RER)

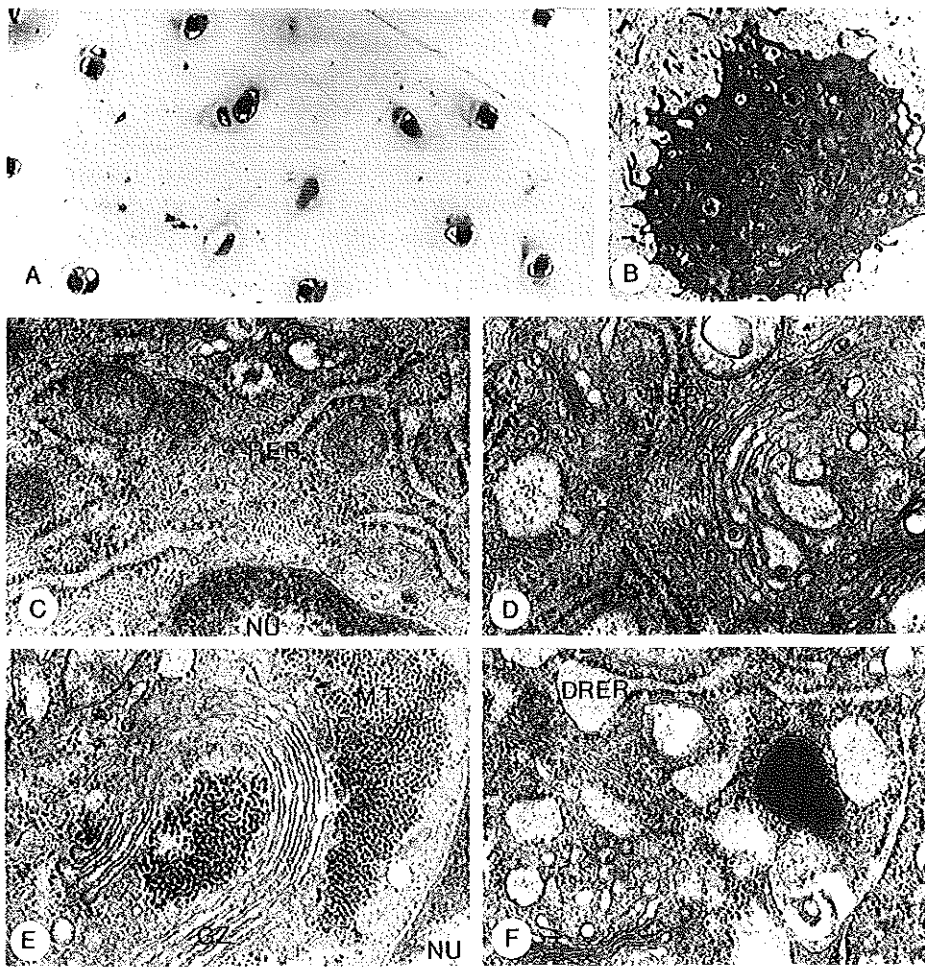
D Cytoplasm of control cell with rough endoplasmic reticulum (RER) and Golgi zone (GZ)

E Golgi zone (GZ) of MED cell and area with many microfilaments (MT) close to nucleus (NU)

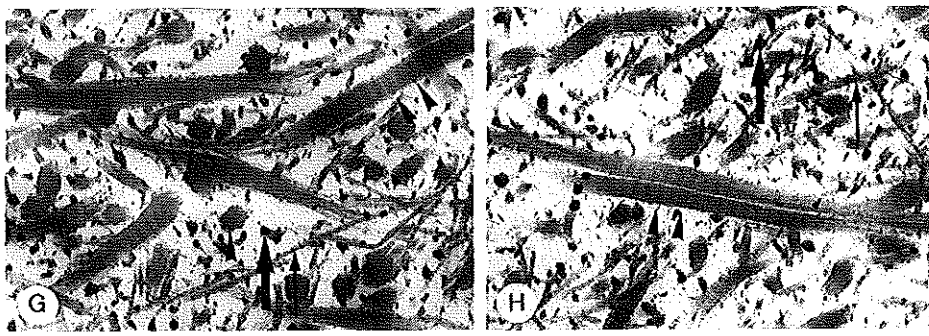
F Dilated rough endoplasmic reticulum (DRER) in MED cartilage

G and H Matrix components of MED (G) and control cartilage (H). Notice characteristic thin (small arrows), thick collagen bundles (arrow heads) and aggregated proteoglycan complexes (thick arrows)

C–H (× 45.000)



see for Figure 6.1.G and 6.1.H next page



6.2 Methods and Materials

During nine surgical procedures on six patients (cases III-6, III-33, IV-4, IV-14, V-18 and IV-81) in a ten year period, either open surgical or arthroscopic biopsies were obtained. In the arthroscopic procedures, a Yamshidi needle biopsy (14 gauge) was taken from the cartilage of the non-weight-bearing part of the medial femoral condyle. In the case of an arthrotomy, biopsies were taken with a Yamshidi needle, or with an osteotome. Consent was obtained from the patients or their parents, and from two healthy individuals (operated for trauma of the elbow or knee) to obtain cartilage biopsies for comparative examination.
The biopsies were fixed 1-2 days in 0.1 M phosphate-buffered (pH 7.4) 2% glutaraldehyde. Postfixation was for two hours in 1% OsO_4 in 0.1 M phosphate buffer (pH 7.4), washing in the buffer, followed by dehydration and embedding in Epon 812. After polymerization, specimens were cut on a Reichert Ultracut E as to obtain semithin (2 μm) and ultrathin (80 nm) sections of both the superficial and deeper zones of the cartilage. Semithin sections were stained with toluidine blue. Ultrathin sections were mounted in identical orientation, stained with uranylacetate (30 min) and lead citrate (10 min) and examined in a Philips 301 electron microscope.
H.M. Peters, Stichting PAMM, Eindhoven and P. Buma, University Hospital Radboud, Nijmegen performed light microscopy. Electron microscopy was by P. Buma, Nijmegen, and W.R. Wilcox, UCLA School of Medicine, Los Angeles.

6.3 Results

6.3.1 Macroscopy

Specimens of joints of cases III-6, III-33, IV-4 and IV-81 showed synovitis and macroscopic degenerative cartilage: the cartilage was irregular, fissured and fibrillated. Loose bodies were removed in three individuals (elbow or knee), and these had a smooth surface. In the two young cases (cases IV-14 and V-18), at arthroscopy of the knee joint, normal joint surfaces were seen.

Postoperative range of motion returned to normal in five/six cases after several months. The locking complaints resolved upon removal of the loose bodies. The MCP II joint mobility in IV-81 remained limited.

6.3.2 *Microscopical Examination*

The three corpora libera (loose bodies) from the elbows of cases III-6, III-33, and IV-4 showed normal cortical bone. The cartilage of the youngest patient (case IV-4) was completely normal. The loose bodies of the two older cases (III-6 and III-33), as well as the MCP joint (IV-81) showed degenerative changes, comparable with osteoarthritis: the zonal organization was less well defined, there was fibrillation and disruption of the superficial layers, and the matrix had a fibrous appearance.
The articular cartilage of the younger cases IV-14 and V-18 *(Figure 6.1.A)* showed normal cartilage: the chondrocytes had a normal appearance and distribution, according to their layer (superficial, transitional and middle layer), and the tidemark was intact; the proteoglycans in the superficial and transitional zones stained normal. Some clustering of chondrocytes was observed, but gross degeneration characterized by chondrocyte death, depletion of proteoglycans, or fibrillation was lacking.

6.3.3 *Electron Microscopy of Cartilage*

This was done in four cases (IV-4, IV-14, IV-81 and V-18) and two controls. The chondrocytes were normal in MED *(Figure 6.1.B)* and in controls, in respect of thin cytoplasmic processes penetrating into the layer of pericellular matrix, cell organelles, and Golgi zones or areas with microfilaments *(Figure 6.1.C, Figure 6.1.D* and *Figure 6.1.E)*. Identically, the rough endoplasmic reticulum showed secretion filled dilatations (case IV-14) *(Figure 6.1.F)*.
The matrix was normal in MED cases and controls with thin and thick collagen fibres, and proteoglycans *(Figure 6.1.G* and *Figure 6.1.H)*. The matrix contained large, banded collagen fibrils and aggregated fibrils.

6.4 Discussion

The present family adolescents have similar arthroscopic findings as reported: in pre-adolescents cartilage is normal, but from adolescence onwards early degenerative changes and loose bodies may occur [Lehman and Murray 1981], as was seen at arthrotomy in cases III-6, III-33, IV-4 and IV-81.
Paucity of chondrocytes in all layers [Anderson et al. 1962] of the growth plate was not observed, nor was irregularity of the epiphyseal plates, or disorganisation of chondrocyte columns. The degenerative changes, observed in the loose bodies of cases III-3 and III-6, probably have a mechanical cause. We have no explanation for the degeneration of the articular cartilage of case IV-81.

Similarly, the 'fingerprint' endoplasmic reticulum described before in MED and pseudoachondroplasia, was not seen in electron microscopical examination in our cases [Maynard et al. 1972, Stanescu et al. 1975, 1982, 1993]. Both mild and severe PSACH as well as MED type-I proved to be linked to the same region of chromosome 19 [Briggs et al. 1993, Hecht et al. 1993, Oehlmann et al 1994]. The gene in this region of chromosome 19p3.1 is coding for COMP (cartilage oligomeric matrix protein), one of the structural proteins of the cartilage extracellular matrix [Briggs et al. 1995, Cohn et al. 1996]. It is assumed that the mutant non-collagenous protein might be stored in the enlarged endoplasmic reticulum in patients with EDM1 and PSACH. In our family the EDM1 locus was excluded *(Chapter 3)*. Thus, production and storage of mutant COMP is very unlikely in our family.
EDM2 is caused by a mutation in the gene coding for the α-2 polypeptide chain of type-IX collagen. Type-IX collagen is located on the surface of collagen type-II fibrils [Eyre 1991]. We had therefore expected to observe abnormalities in the collagen fibrils, but we did not find any abnormalities. Possible explanations for the lack of inclusion bodies and the normal collagen fibrils are: 1) the abnormal type-IX collagen is efficiently secreted; 2) the mutant COL9A2 gene is not expressed; 3) there is little type-IX collagen in the hyaline cartilage and the system in the rough endoplasmic reticulum for clearing out abnormal proteins is efficient enough to prevent retention; 4) the abnormal α-2 chains are rapidly destroyed and not incorporated into trimers; or 5) the mRNA for the mutant allele is unstable and rapidly degraded. Based on our findings we cannot elucidate the mechanism; further investigations, such as biochemical studies on cartilage and cultured chondrocytes of affected individuals, are indicated to find the mechanism.

6.5 CONCLUSIONS

From this study it may be concluded that:

- Articular cartilage in young patients from this family with MED has a normal macroscopical and microscopical appearance.
- Cartilage of elderly patients with MED shows degenerative changes similar to osteoarthritis.
- At electron microscopy, the rough endoplasmic reticulum of cartilage cells as well as collagen fibrils are normal in this type of MED.

7 Conclusions

7.1 Nomenclature

Classification of the osteochondrodysplasias has a long tradition, since 1951. The initial three groups [Rubin 1964], became extended into the Paris Nomenclature of Skeletal Dysplasias, based on clinical and radiographical findings. As it is extensive, it is less easily introduced into clinical practice. Moreover, it is refined every two years at the International Skeletal Dysplasia Meetings *(Chapter 10)*. Due to developments in genetics since the 1980's, future classifications will be based more on the chromosomal localisation of the particular genes involved. The genes involved in several skeletal dysplasias and connective tissue disorders have been identified [Dietz and Mathews 1996]. The number of chondrodysplasia loci, however, is much smaller than predicted a decade ago. Mutations of COL2A1, for instance, cause at least six different phenotypes [Vikkula et al. 1994, Horton 1995].
The phenotypic differentiation into a more severe Fairbank type [1947] and a milder Ribbing type [1937], introduced confusion, because of both intrafamilial variability and alternating application of these eponyms on either severe or mild types [Ballo et al 1997, Kozlowski and Lipska 1967, Lachman et al. 1973]. It might be better to use MED type I or II, or, according to the genetic loci, resp. EDM1 and EDM2.

7.2 Clinical Presentation and Incidence

The family presented has multiple epiphyseal dysplasia, with extremely variable expression for site and severity of the lesions, and number of joints (knees, ankles, elbows and hands) involved. No straightforward explanations for this variation were found in weight, height, sex, or height of affected relatives.
The single large family studied influenced the prevalence of MED in our region. The estimated prevalence of 11.2 to 16.3 per million [Wynne-Davies and Gormley 1985] is probably higher [Andersen and Hauge 1989, Jacobs 1968, Mena and Pearson 1976].

7.3 Genetic Studies

Mutations of either of the two known loci for MED (EDM1 and EDM2) seem to be associated with different genetic defects and phenotypes. MED type-I, caused by a mutation in the cartilage oligomeric matrix protein (COMP) gene, is associated with a more severe MED with hip involvement [Weaver et al. 1993]. MED type-II, caused by a mutation in the COL9A2 gene, has a moderate phenotype, without hip involvement (the present family, and family A of Barrie et al. [1958]). The absence of hip

and shoulder involvement in MED II is unexplained. One possibility might be the different contributions of COL9A2 to these joints. Further research in families with MED is necessary to establish the range of genetic and clinical heterogeneity of these disorders.

7.4 Radiographic Studies

A precise diagnosis of MED II is possible now by mutation analysis. However, mutation analysis is expensive and time consuming. In our family, radiographic anthropometry was a very useful method for diagnosis. So, the initial diagnostic work-up in a family with MED type II has to include physical examination and standard radiographs of the knee joint in children aged 4 to 15 years old in order to detect the disease. Supplemental radiographic studies were not contributing. Our arthroscopic observations in two cases are concordant with the findings on their MRI scans. Thus, no abnormalities were seen at the articular cartilage, neither at arthroscopy, nor on MRI scans *(Chapter 5)*.

7.5 Pathological Findings

In the MED type II family studied here, microscopy of the cartilage was normal until young adulthood and unspecific arthritic changes were seen in the older cases. Disorganisation of cartilage cell columns, and structurally abnormal abundant intercellular cartilage matrix with cleft formations and areas of degeneration was reported [Anderson et al. 1962]. Another microscopical study showed ovoid or rounded chondrocytes, with large vacuoles, and a 'fingerprint' endoplasmic reticulum [Maynard et al. 1972, Stanescu et al. 1975, 1982, 1993]. The most likely explanation for these differences is, that these other studies were performed on patients with MED type I; here one may expect accumulation of non collagenous protein material (probably mutant COMP) in the rough endoplasmic reticulum, the inclusion bodies. In MED type-II, caused by a mutation in the gene coding for the α2 polypeptide chain of type-IX collagen, abnormalities in the collagen fibrils were expected, but not found. Apparently, there is little type-IX collagen in the hyaline cartilage of the examined adolescent and adult patients, whereas there is efficient clearance of abnormal proteins by the rough endoplasmic reticulum. Other explanations are that the abnormal α-2 chains are rapidly destroyed and not incorporated into trimers; that the abnormal type-IX collagen is efficiently secreted; that the mutant COL9A2 gene is not expressed in the articular hyaline cartilage; or that the mRNA for the mutant allele is unstable and rapidly degraded.

Another unsolved problem is the beginning of complaints during childhood, and their decrease during adolescence, unless the joints show either marked axial deviations or osteoarthritis. This may be explained by the physiologic decrease of collagen IX during development: in fetal cartilage it accounts for up to 10% of the collagen, and for only 1-2% in adult hyaline cartilage [Eyre et al. 1987]. This decrease in synthesis starts in fetal life [Reginato et al 1995]. Collagen IX probably functions in binding and stabilising the type II collagen meshwork [Bruckner at al. 1988, Smith

and Brand 1992]. So, collagen IX has its most important role during the first years of life. In this way, it can be explained that complaints develop on increasing weight bearing of the epiphyses and disappear after complete calcification of the epiphyses. This fact can also explain the 'constitutionally weak epiphysis' described in MED [Ribbing 1937].

7.6 The Effects of Mutant COL9A2

The disturbance of calcification of the epiphysis, in contrast to the relatively normal articular cartilage, has not (yet) been explained. The calcification of the cartilage matrix is clearly a complex interactive process. It has a number of important players, whose roles are starting to become clear. The uncalcified extracellular matrix of the growth plate is composed of a fibrillar network of type II collagen; these collagen fibrils are much thinner than those found in adult hyaline cartilage, destined to survive [Poole et al. 1989]. Collagen IX, covalently cross-linked to type II collagen, may play an important intermediary role in the interaction of other molecules with the fibrillar network of type II collagen. Likewise, collagen IX may function as a regulator of fibril diameter [Wotton et al. 1988]. It can therefore be theorized that mutant collagen IX causes the disturbed calcification of the epiphysis. This can lead to deformation of the epiphysis, axial deformities and mechanical osteoarthritis. On the other hand, due to its supramolecular function, mutant collagen IX may alter the physical integrity of articular cartilage; in this way, it might be one of the causes of osteoarthritis after adolescence.
The specific localisation of involved joints in MED type II and the absence of shoulder and hip involvement is in striking contrast with MED type I. This might be studied by cartilage analysis of hip and shoulder joints in patients with MED type II, and differential quantification of normal and mutant IXα2 chains.

Summary

Multiple Epiphyseal Dysplasia (MED) is one of the genetic osteochondrodysplasias. It affects both sexes. Autosomal dominant inheritance is the main form of inheritance, although autosomal recessive inheritance and spontaneous mutations have been described. MED is characterised by painful joints and gait abnormalities. Onset of the disease is early childhood. Radiographs show delayed and irregular mineralization of epiphyseal ossification centers and centers for carpal and tarsal bones. The disease may cause axial deformities of the limbs. Vertebral bodies sometimes show mild aberrations such as anterior wedging.
Pathological studies have revealed an irregularity of the epiphyseal line. Chondrocytes are decreased in number; areas of matrix between cartilage columns show degenerative changes. The treatment of MED is symptomatic.

The phenotypic spectrum of MED in a large family in the South-East region of the Netherlands was studied. In this family, 54 individuals have involvement of the knee, elbow, ankle, wrist, hand and feet joints. No shoulder or hip involvement is ever seen after adolescence. The height of the affected individuals is short to normal; spinal involvement is never seen. The penetrance of the genetic defect is complete. However, the expression of the genetic defect is extremely variable; there are no correlations with body height or sex *(Chapter 2)*.

At least three loci have been suggested for MED. The first known locus for MED (EDM1) has been mapped to a region on chromosome 19. The second locus (EDM2) was mapped to the short arm of chromosome 1. Exclusion of the EDM1 and EDM2 loci in other families with MED suggests the existence of at least one additional locus. Genetic linkage studies were performed in the present family, and the EDM1 locus was excluded. However, there was a significant linkage between one of the markers for the EDM2 locus (MYCL) and the disorder *(Chapter 3)*. The maximum lod score for MYCL proved to be Z_{max}=15.31 at Θ = 0.016.

Linkage analysis was performed before. The mutation of the gene, however, was not yet detected. By using RNA from affected chondrocytes, we were able to detect the mutation of the involved gene *(Chapter 4)*. This mutation was in the region of chromosome 1 containing a type IX collagen gene (COL9A2), one of the cartilage specific collagen types. The study revealed that the sequence of the 5′ splice site of intron 3, GTGAG, was converted to GCGAG in one allele of an affected patient. The mutation in the COL9A2 gene leads to an inframe deletion of 12 amino acids in the collagen IXα2 chain. All affected individuals, including the one who was recombinant for MYCL, were heterozygous for this change. The lod score for this mutation was Z=17.55 at recombination fraction θ=0.0.

Anthropometric measurements were made of the distal femoral metaphysis and epiphysis of the knee joint of 15 individuals (12 affected, three not affected). Epiphyseal height was plotted against the width of both the epiphysis and the metaphysis. In 11 of 12 individuals with MED, the plotted values were more than two standard deviations below the mean. Sensitivity was 92%, specificity was 100%, and positive predictive value was 100%. So, anthropometry proved to be useful to detect involvement, with a high positive predictive value *(Chapter 5)*.
MRI investigations of the knee joints were performed in six young individuals with MED. As in conventional radiographs, MRI images showed irregular and delayed ossification of the epiphyses; articular cartilage had a normal appearance. We conclude that MRI investigations had no additional value in confirming the diagnosis, nor in planning treatment. The indications for MRI in patients with MED are the same as in patients without this condition.

Microscopical studies, performed on articular cartilage, obtained after operations on six patients with MED, have been described in *Chapter 6*. Arthroscopy in two young patients showed a normal appearance of the cartilage; arthrotomy in the other four patients showed degenerative cartilage. Light microscopical examination revealed no abnormalities in three young patients, whereas cartilage of three other patients showed degenerative changes, as can be seen in osteoarthritis. Electron microscopical examination of the chondrocytes showed normal ultrastructural characteristics. The matrix contained large, banded collagen fibrils, aggregated fibrils and proteoglycans. The collagen fibrils were variable in size and showed a normal appearance and distribution. These findings are not concordant with the literature; however, these reported cases probably had MED type I, whereas our patients have MED type II.

In *Chapter 7* the overall findings of this thesis are discussed. Based on the literature and our findings, we assume that the two known loci for MED (EDM1 and EDM2) have their own phenotypes. We recommend using the names MED type I or II, instead of the names Fairbank or Ribbing Type formerly used. Besides, we advise performing physical examination and standard radiographs of the knee joint in children with possible MED. Finally, the normal pathological findings are discussed, as well as the possible effects of the mutant COL9A2. Collagen IX possibly has its most important function during the first years if life. Mutant collagen IX may cause disturbed calcification of the epiphysis, leading to the delayed and fragmented ossification, seen on radiographs.

9 Samenvatting

Multipele epiphysaire dysplasie (MED) is een van de osteochondrodysplasieën; het is een erfelijke ziekte en komt zowel bij mannen als bij vrouwen voor. Overerving is autosomaal dominant, alhoewel ook autosomaal recessieve overerving en spontane mutaties zijn beschreven. De ziekte wordt gekenmerkt door pijnlijke gewrichten en/of een afwijkend looppatroon. De eerste ziekteverschijnselen beginnen in de vroege jeugd. Op röntgenfoto's is een vertraagde en onregelmatige verbening van groeikernen van lange pijpbeenderen en middenhands- en middenvoetsbeenderen te zien. Dit kan leiden tot asafwijkingen van de extremiteiten. Soms is geringe wigvorming van de wervels te zien.
Pathologisch-anatomische onderzoekingen toonden aan dat sprake is van een irregulaire groeischijf, een vermindering van het aantal chondrocyten, en degeneratieve veranderingen van de matrix tussen kraakbeencellen. De behandeling van MED is symptomatisch.

De klinische en radiologische gegevens van een familie met MED uit de directe omgeving van het Sint Joseph Ziekenhuis te Veldhoven worden beschreven in *hoofdstuk 2*. Van deze familie hebben 54 leden afwijkingen aan hun knieën, hun elleboog-, enkel-, pols- en handgewrichten of hun voet. Bij de volwassenen zijn geen afwijkingen vastgesteld aan heup- of schoudergewrichten. Evenmin zijn afwijkingen gezien aan de wervelkolom. De lichaamslengte van aangedane familieleden is normaal of kleiner dan normaal. De penetrantie van het gen is volledig, maar de expressie ervan is uiterst variabel. Er is evenwel geen relatie tussen de ernst van de ziekte en de lichaamslengte of het geslacht.

Er zijn ten minste drie loci voor MED. Het locus dat het eerst ontdekt is (EDM1) ligt op chromosoom 19. Het tweede locus (EDM2) ligt op de korte arm van chromosoom 1. Er zijn families met MED bij wie geen koppeling gevonden is met een van deze twee loci; er moet dus ten minste nog een derde locus zijn. In de door ons bestudeerde familie is het EDM1-locus uitgesloten. Er was wel significante koppeling tussen de ziekte en een marker in de buurt van het EDM2-locus, het MYCL-locus *(hoofdstuk 3)*. De maximale LOD-score was 15,31 bij een recombinantiefractie van 0,016.

Het gevonden locus was reeds langer bekend; de mutatie zelf was echter nog steeds niet gevonden. Wij waren in staat moleculair genetische onderzoeken naar het aangedane gen te verrichten door RNA te isoleren uit chondrocyten van een patiënte met MED *(hoofdstuk 4)*. De genmutatie bij de onderzochte vorm van MED is aanwezig in het gedeelte van chromosoom 1, waar ook een der collageen IX-genen (COL9A2) ligt; collageen IX is een van de voor kraakbeen specifieke collagenen. Uit onze studie bleek, dat de volgorde van de baseparen aan het 5' eind van intron 3 in een allel

van de patiënte veranderd was: GCGAG in plaats van GTGAG. Door deze mutatie in het COL9A2-gen ontstaat een tekort van 12 aminozuren in de collageen IXα2-keten. Alle aangedane individuen, inclusief degene die recombinant bleek te zijn voor het MYCL-gen, hebben de mutatie. De LOD-score voor deze mutatie was 17,55 bij een recombinatiefractie van 0,0.

Radiologische studies, te weten anthropometrie- en MRI-studies, worden besproken in *hoofdstuk 5*. Bij vijftien kinderen (twaalf met MED, drie niet aangedaan) zijn metingen verricht aan de distale epifyse en metafyse van het femur op standaard voor-achterwaartse röntgenfoto's van het kniegewricht. De hoogte van de epifyse werd grafisch uitgezet tegen de breedte van de epifyse en de metafyse. Bij elf van de twaalf personen met MED bleken deze waarden meer dan twee standaarddeviaties onder het gemiddelde te zijn. De sensitiviteit was 92%, de specificiteit 100% en de positief voorspellende waarde was eveneens 100%. Radiologische anthropometrie is dus zeer bruikbaar om bij jonge kinderen van de onderzochte familie MED aan te tonen.
MRI-studies zijn verricht bij zes jonge mensen met MED. Net als op conventionele röntgenfoto's tonen MRI-beelden irregulaire en vertraagde verbening van de epifyses. Het gewrichtskraakbeen had een normaal aspect. Wij concluderen dan ook, dat MRI geen aanvullende waarde heeft om de diagnose MED te stellen.

Microscopische studies, die verricht zijn aan het gewrichtskraakbeen, verkregen bij verschillende operaties bij patiënten met MED, worden beschreven in *hoofdstuk 6*. Het kraakbeen bij jongere patiënten had een normaal aspect bij arthroscopie. Degeneratief kraakbeen werd gezien bij arthrotomie bij oudere patiënten. Licht microscopisch onderzoek toonde geen afwijkingen aan bij jonge mensen; kraakbeen onderzoek bij twee oudere patiënten toonde degeneratieve afwijkingen, zoals deze ook worden gezien bij arthrose. Elektronenmicroscopisch onderzoek leverde normale beelden op wat betreft de chondrocyten. De intercellulaire matrix bevatte proteoglycanen en grote, gestreepte collageenvezels, die soms in groepjes lagen. De collageenvezels hadden een normaal aspect en een normale verdeling over de intercellulaire matrix; de vezels hadden verschillende diktes. Onze bevindingen komen niet overeen met literatuurgegevens; de gepubliceerde onderzoekingen betreffen evenwel waarschijnlijk patiënten met MED type I.

In *hoofdstuk 7* worden de algemene conclusies van dit proefschrift besproken. Zowel op basis van de literatuur als op grond van onze eigen gegevens, menen wij dat de twee bekende loci voor MED (EDM1 en EDM2) ieder hun eigen fenotype hebben. Wij adviseren dan ook in het vervolg de namen MED type I of II te gebruiken, in plaats van de veelal gebruikte namen 'type Fairbank' of 'type Ribbing'. Daarnaast adviseren wij om bij een mogelijk aangedaan kind uit een familie met MED lichamelijk onderzoek te verichten en standaard röntgenfoto's van de kniegewrichten te laten maken.
Ten slotte worden de normale bevindingen bij pathologisch-anatomisch onderzoek besproken, alsmede de mogelijke effecten van het afwijkende collageen IX. Collageen IX heeft waarschijnlijk zijn belangrijkste rol tijdens de eerste levensjaren; hierdoor kan een afwijkend collageen IX aanleiding geven tot een verstoring van de verkalking

van de epifyse. Dit leidt dan weer tot de vertraagde en onregelmatige verbening van de epifyse, zoals dat op röntgenfoto's van patiënten met MED wordt gezien.

10 International Classification of Osteochondrodysplasias (2nd revision)

A DEFECTS OF THE TUBULAR (AND FLAT) BONES AND/OR AXIAL SKELETON

1 ACHONDROPLASIA GROUP
Thanatophoric dysplasia
Thanatophoric dysplasia-straight femur/cloverleaf skull type
Achondroplasia
Hypochondroplasia

2 ACHONDROGENESIS
Type IA
Type IB

3 SPONDYLODYSPLASTIC GROUP (Perinatally lethal)
San Diego type
Torrance type
Luton type

4 METATROPIC DYSPLASIA GROUP
Fibrochondrogenesis
Schneckenbecken dysplasia
Metatropic dysplasia

5 SHORT RIB DYSPLASIA GROUP (WITH/WITHOUT POLYDACTYLY)
SR(P) Type I Saldino Noonan
SR(P) Type II Majewski
SR(P) Type III Verma-Naumoff
SR(P) Type IV Beemer-Langer
Asphyxiating Thoracic Dysplasia
Ellis-van Creveld Dysplasia

6 ATELOSTEOGENESIS/ DIASTROPHIC DYSPLASIA GROUP
Boomerang dysplasia
Atelosteogenesis type 1
Atelosteogenesis type 2 (de la Chapelle)
Omodysplasia I (Maroteaux)
Omodysplasia II (Borochowitz)
Oto-palato-digital syndrome type 2
Diastrophic dysplasia
Pseudodiastrophic dysplasia

7 KNIEST-STICKLER DYSPLASIA GROUP
Dyssegmental dysplasia-Silverman Handmaker type
Dyssegmental dysplasia-Rolland-Desbuqois type
Kniest dysplasia
Oto-spondylo-megaepiphyseal dysplasia
Stickler dysplasia (heterogeneous, some not linked to COL2A1)

8 SPONDYLOEPIPHYSEAL DYSPLASIA CONGENITA GROUP
Langer-Saldino Dysplasia (Achondrogenesis type II)
Hypochondrogenesis
Spondyloepiphyseal dysplasia congenita

9 OTHER SPONDYLO EPI-(META-)PHYSEAL DYSPLASIAS
X-linked Spondyloepiphyseal dysplasia tarda
Other late onset Spondylo epi-(meta-)physeal dysplasias
Progressive pseudorheumatoid dysplasia
Dyggve-Melchior-Clausen dysplasia
Wolcott-Rallison dysplasia
Immunoosseous dysplasia
Pseudoachondroplasia
Opsismodysplasia

10 DYSOSTOSIS MULTIPLEX GROUP
Mucopolysaccharidosis type I-H
Mucopolysaccharidosis type I-S
Mucopolysaccharidosis type II
Mucopolysaccharidosis type III-A
Mucopolysaccharidosis type III-B
Mucopolysaccharidosis type III-C
Mucopolysaccharidosis type III-D
Mucopolysaccharidosis type IV-A
Mucopolysaccharidosis type IV-B
Mucopolysaccharidosis type VI
Mucopolysaccharidosis type VII
Fucosidosis
α-Mannosidosis
β-Mannosidosis
Aspartylglucosaminuria
gM1 Gangliosidosis, several forms
Sialidosis, several forms
Sialic storage disease
Galactosialidosis, several forms
Mucosulfatidosis
Mucolipidosis II
Mucolipidosis III
Mucolipidosis IV

11 SPONDYLOMETAPHYSEAL DYSPLASIAS
Spondylometaphyseal dysplasia-Kozlowski type
Spondylometaphyseal dysplasia-corner fracture type (Sutcliffe)
Spondyloenchondrodysplasia

12 EPIPHYSEAL DYSPLASIAS
Multiple epiphyseal dysplasia Fairbanks/Ribbing

13 CHONDRODYSPLASIA PUNCTATA (STIPPLED EPIPHYSES) GROUP
Rhizomelic type
Conradi-Hünermann type
X-linked recessive type
MT-type
Others including CHILD syndrome, Zellweger syndrome, Warfarin embryopathy, chromosomal abnormalities, fetal alcohol syndrome

14 METAPHYSEAL DYSPLASIAS
Jansen type
Schmid type
Spahr type
McKusick type (CHH)
Metaphyseal Anadysplasia
Shwachmann type
Adenosine deaminase deficiency

15 BRACHYRACHIA (SHORT SPINE DYSPLASIA)
Brachyolmia, several types

16 MESOMELIC DYSPLASIAS
Dyschondrosteosis
Langer type
Nievergelt type
Robinow type

17 ACRO/ACRO-MESOMELIC DYSPLASIAS
Acromicric dysplasia
Geleophysic dysplasia
Acrodysostosis
Tricho-rhino-phalangeal dysplasia type 1
Tricho-rhino-phalangeal dysplasia type 2
Saldino-Mainzer dysplasia
Pseudohypoparathyroidism, several types
Cranioectodermal dysplasia
Acromesomelic dysplasia
Grebe dysplasia

18 DYSPLASIAS WITH SIGNIFICANT (BUT NOT EXCLUSIVE) MEMBRANEOUS BONE INVOLVEMENT
Cleidocranial dysplasia
Osteodysplasty, Melnick-Needles

19 BENT BONE DYSPLASIA GROUP
Campomelic dysplasia
Kyphomelic dysplasia
Stüve-Wiedemann dysplasia

20 MULTIPLE DISLOCATIONS WITH DYSPLASIAS
Larsen syndrome
Desbuquois syndrome
Spondylo-epi-metaphyseal dysplasia with joint laxity

21 OSTEODYSPLASTIC PRIMORDIAL DWARFISM GROUP
Type 1
Type 2

22 DYSPLASIAS WITH DECREASED BONE DENSITY
Osteogenesis Imperfecta (several types)
Osteoporosis with pseudoglioma
Idiopathic Juvenile Osteoporosis
Bruck syndrome
Homocystinuria
Singleton-Merten syndrome
Geroderma Osteodysplastica
Menkes syndrome

23 DYSPLASIAS WITH DEFECTIVE MINERALIZATION
Hypophosphatasia
Hypophosphatemic rickets
Pseudodeficiency rickets, several types
Neonatal hyperparathyroidism

24 DYSPLASIAS WITH INCREASED BONE DENSITY
Osteopetrosis
- a precocious type
- b delayed type
- c intermediate type
- d with renal tubular acidosis

Dysosteosclerosis
Pycnodysotosis
Osteosclerosis-Stanescu type
Axial osteosclerosis including
- a Osteomesopycnosis
- b with Bamboo hair (Netherton syndrome)
- c Tricho-thiodystrophy

Osteopoikilosis
Melorheostosis
Osteopathia Striata
Osteopathia Striata with cranial sclerosis
Diaphyseal dysplasia, Camurati-Engelmann
Craniodiaphyseal dysplasia
Lenz-Majewski dysplasia
Craniometaphyseal dysplasia
Endosteal hyperostosis
- a van Buchem disease
- b Sclerosteosis
- c Worth disease
- d with cerebellar hypoplasia

Pachydermoperiostosis
Fronto-metaphyseal dysplasia
Craniometaphyseal dysplasia
- a severe type
- b mild type

Pyle (disease) dysplasia
Osteoectasia with hyperphosphatasia
Oculo-dento-osseous dysplasia
- a severe type
- b mild type

Familial Infantile Cortical Hyperostosis-Caffey

B DISORGANIZED DEVELOPMENT OF CARTILAGENOUS AND FIBROUS COMPONENTS OF THE SKELETON

Dysplasia epiphysealis hemimelica
Multiple cartilaginous exostoses
Enchondromatosis (Ollier)
Enchondromatosis with hemangiomata (Maffucci)
Metachondromatosis
Osteoglophonic dysplasia
Fibrous dysplasia (Jaffe-Lichtenstein)
Fibrous dysplasia with pigmentary skin changes and precocious puberty (McCune-Albright)
Cherubism
Myofibromatosis (generalized fibromatosis)

C IDIOPATHIC OSTEOLYSES

1 Predominantly phalangeal
  Hereditary acroosteolysis several forms
  Hajdu-Cheney type
2 Predominantly carpal/tarsal
  Carpal-tarsal osteolysis with nephropathy
  Francois Syndrome (dermo-chondro-corneal dystrophy)

3 Multicentric
Winchester syndrome
Torg type
Mandibulo-acral dysplasia
4 Other
Familial expansile osteolysis

From: Beighton et al. 1992

11 References

Ala-Kokko L, Baldwin CT, Moskowitz RW, Prockop DJ. Single base mutation in the type II procollagen gene (COL2A1) as a cause of primary osteoarthritis associated with a mild chondrodysplasia. Proc Natl Acad Sci USA 1990;87:6565-6568.

Amir D, Mogle P, Weinberg H. Multiple epiphyseal dysplasia in one family. A further review of seven generations. J Bone Joint Surg 1985;67B:809-813.

Andersen PE, Schantz K, Bollerslev J, Justesen P. Bilateral femoral head dysplasia and osteochondritis. Multiple epiphyseal dysplasia tarda, spondylo-epiphyseal dysplasia tarda, and bilateral Legg-Perthes disease. Acta Radiol 1988;29:705-709.

Andersen PE, Hauge M. Congenital generalised bone dysplasias: a clinical, radiological, and epidemiological survey. J Med Genet 1989;27:37-44.

Anderson CE, Crane JT, Harper HA, Hunter TW. Morquio's disease and dysplasia epiphysialis multiplex. J Bone Joint Surg 1962;44A:295-306.

Apley AG, Solomon L. Apley's System of Orthopaedics and Fractures, 7th edition. Oxford: Butterworth-Heinemann Ltd, 1993.

Ballo R, Briggs MD, Cohn DH, Knowlton RG, Beighton PH, Ramesar RS. Multiple epiphyseal dysplasia, Ribbing type: A novel point mutation in the COMP gene in a South African family. Am J Med Genet 1997;68:396-400.

Barrie H, Carter C, Sutcliffe J. Multiple epiphysial dysplasia. Br Med J 1958;2:133-137.

Barrington-Ward LE. Double coxa vara with other deformities occurring in brother and sister. Lancet 1912;1:157-159.

Beals RK. Tricho-rhino-phalangeal dysplasia. J Bone Joint Surg 1973;55A:821-826.

Beighton P, Goldberg L, Op't Hof J. Dominant inheritance of multiple epiphyseal dysplasia, myopia and deafness. Clin Genet 1978;14:173-177.

Beighton P, Giedion A, Gorlin R, Hall J, Horton B, Kozlowski K, Lachman R, Langer LO, Maroteaux P, Poznanski A, Rimoin DL, Sillence D, Spranger J. International classification of osteochondrodysplasias. Am J Med Genet 1992;44:223-229.

Bercu G. Facteur familial dans la dysplasie poly-epiphysaire. J Belge Radiol 1971; 54:541.

Berg PK. Dysplasia epiphysialis multiplex: a case report and review of the literature. Am J Roentgenol 1966;97:31-38.

Bettmann E. Beobachtungen über Hüftgelenksveränderungen bei 19 Familienangehörigen. Z Orthop 1932;53:327-344.

Bhowmick SK, Kaste SC. Multiple epiphyseal dysplasia misdiagnosed as bioinactive growth hormone disorder. South Med J 1991;84:1259-1262.

Briggs MD, Rasmussen IM, Weber JL, Yuen J, Reinker K, Garber AP, Rimoin DL, Cohn DH. Genetic linkage of mild pseudoachondroplasia (PSACH) to markers in the pericentromeric region of chromosome 19. Genomics 1993;18:656-660.

Briggs MD, Choi H, Warman ML, Loughlin JA, Wordsworth P, Sykes BC, Irven CMM, Smith M, Wynne-Davies R, Lipson MH, Biesecker LG, Garber AP, Lachman R, Olsen BR, Rimoin DL, Cohn DH. Genetic mapping of a locus for multiple epiphyseal dysplasia (EDM2) to a region of chromosome 1 containing a type IX collagen gene. Am J Hum Genet 1994;55:678-684.

Briggs MD, Hoffman SMG, King LM, Olsen AS, Mohrenweiser H, Leroy JG, Mortier GR, Rimoin DL, Lachman RS, Gaines ES, Cekleniak JA, Knowlton RG, Cohn DH. Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene. Nat Genet 1995;10:330-336.

Bruckner P, Mendler M, Steinmann B, Huber S, Winterhalter KH. The structure of human collagen type IX and its organization in fetal and infant cartilage fibrils. J Biol Chem 1988;263:16911-16917.

Brunzlow H, Loreck D, Trzenschik K. Beitrag zur Multiplen Epiphysealen Dysplasie. Radiol Diagn Berl 1988;29:571-580.

Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-159.

Christensen WR, Lin RK, Berghout J. Dysplasia epiphysalis multiplex. Am J Roentgenol Radium Ther Nucl Med 1955;74:1059-1067.

Clark WA. Multiple epiphyseal dystrophy: case report. J Bone Joint Surg 1929:11:566-569.

Coates CJ, Williamson DM, Menelaus MB. Vertical subluxation of the patella: an unusual feature of multiple epiphyseal dysplasia. Aust N Z J Surg 1992:62:164-165.

Cohen MM, Kreiborg S. Skeletal abnormalities in the Apert syndrome. Am J Med Genet 1993;47:624-632.

Cohn DH, Briggs MD, King LM, Rimoin DL, Wilcox WR, Lachman RS, Knowlton RG. Mutations in the cartilage oligomeric matrix protein (COMP) gene in pseudoachondroplasia and multiple epiphyseal dysplasia. Ann N Y Acad Sci 1996; 785:188-194.

Cowan DJ. Multiple epiphysial dysplasia. Br Med J 1963;2:1629.

Crossan JF, Wynne-Davies R, Fulford GE. Bilateral failure of the capital femoral epiphysis: bilateral Perthes disease, multiple epiphyseal dysplasia, pseudoachondroplasia, and spondyloepiphyseal dysplasia congenita and tarda. J Pediatr Orthop 1983;3:297-301.

Cruz-Conde R, Amaya S, Valdivia P, Hernández M, Calvo M. Dysplasia epiphysealis hemimelica. Case Report. J Pediatr Orthop 1984;4:625-629.

Daentl DL, Siegel RC, Nevo Z, Scheck M, Parker JM, Smith G, Sakovich L, Ashley RK, Larsen LJ. Auriculoepiphyseal dysplasia (multiple epiphyseal dysplasia and anomalous auricles): Clinical, structural and biochemical studies. Birth Defects 1975;11:217-226.

Dahners LE, Francisco WD, Halleran WJ. Findings at arthrotomy in a case of double layered patellae associated with multiple epiphyseal dysplasia. J Pediatr Orthop 1982;2:67-70.

Dandee DJ. Editorial. Arthroscopy and MRI for the knee. J Bone Joint Surg 1997; 79B:520.

Deere M, Halloran Blanton S, Scott CI, Langer LO, Pauli RM, Hecht JT. Genetic heterogeneity in multiple epiphyseal dysplasia. Am J Hum Genet 1995;56:698-704.

Diamond LS. Natural history and treatment of early hip disease in spondylo- and multiple epiphyseal dysplasia. J Bone Joint Surg 1973;55A:1318.

Dietz FR, Mathews KD. Update on the genetic bases of disorders with orthopaedic manifestations. J Bone Joint Surg 1996;78A:1583-1598.

Elsbach L. Bilateral hereditary micro-epiphysial dysplasia of the hips. J Bone Joint Surg 1959;41B:514-523.

Emr J. Dysplasia epiphysialis multiplex occuring in two brothers. Acta Chir Orthop Traum Cech 1952;19:310-316.

Eyre DR, Apon S, Wu JJ, Ericsson LH, Walsh KA. Collagen type IX: evidence for covalent linkages to type II collagen in cartilage. FEBS Lett 1987;220:337-341.

Eyre DR. The collagens of articular cartilage. Semin Arthritis Rheum 1991;21:2-11.

Erlebacher A, Filvaroff EH, Gitelman SE, Derynck R. Toward a molecular understanding of skeletal development. Cell 1995;80:371-378.

Fairbank HAT. Generalized diseases of the skeleton. Proc R Soc Med 1935;28:1611-1619.

Fairbank HAT. Dysplasia epiphysealis multiplex. Proc R Soc Med 1946;39:315-317.

Fairbank HAT. Dysplasia epiphysialis multiplex. Br J Surg 1947;34:225-232.

Fässler R, Schnegelsberg PNJ, Dausman J, Shinya T, Muragaki Y, McCarthy MT, Olsen BR, Jaenisch R. Mice lacking α1(IX) collagen develop noninflammatory degenerative joint disease. Proc Nat Acad Sci USA 1994;91:5070-5074.

Felman AH. Multiple epiphyseal dysplasia. Radiology 1969;93:119-125.

Freiberger RH. Multiple epiphyseal dysplasia: A report of 3 cases. Radiology 1958; 70:379-385.

Gamboa I, Lisker R. Multiple epiphyseal dysplasia tarda: A family with autosomal recessive inheritance. Clin Genet 1974;6:15-19.

Garn SM, Leonard WR, Poznanski AK. Applications of the pattern variability index to the quantification of dysmorphogenesis in the hand. Am J Med Genet 1987; 27:143-152.

Geiser M. Die aseptische Knochennekrose als Erbleiden. Schweiz Med Wschr 1954; 84:903-906.

Geissler A, Ott D. Dysplasia epiphysialis multiplex tarda. Unterschiedliche Aussage von MRT und Röntgenbild. Fortschr Röntgenstr 1992;156:100-102.

Gibson T, Highton J. Multiple epiphyseal dysplasia: a family study. Rheumatol Rehabil 1979;18:239-242.

Giedion A, Prader A, Fliegel C, Krasikov N, Langer L, Poznanski A. Angel-shaped phalango-epiphyseal dysplasia (ASPED): identification of a new genetic bone marker. Am J Med Genet 1993;47:765-771.

Goetsch E. Zur Kenntnis der polytopen enchondralen Dysostosen. Fortschr Röntgenstr 1953;79:472-476.

Griffiths HED, Witherow PJ. Perthes' disease and multiple epiphyseal dysplasia. Postgrad Med J 1977;53:464-470.

Grimm J, Just M. Epiphysäre Dysplasie des Hüftgelenks, Diagnostik und Differentialdiagnostik mit der MRT. Fortschr Röntgenstr 1992;157:47-52.

Grudzinski Z. Über eine neue mit Achondroplasie (Chondrodystrophie) verwandte Krankheitsform. Fortschr Röntgenstr 1928;38:873-882.

Hankenson LG, Ozonoff MB, Cassidy SB. Epiphyseal dysplasia with coxa vara, microcephaly, and normal intelligence in sibs: expanded spectrum of Lowry-Wood syndrome? Am J Med Genet 1989;33:336-340.

Hecht JT, Francomano CA, Briggs MD, Deere M, Conner B, Horton WA, Warman M, Cohn DH, Halloran Blanton S. Linkage of typical pseudoachondroplasia to chromosome 19. Genomics 1993;1-8:661-666.

Hecht JT, Nelson LD, Crowder E, Wang Y, Elder FFB, Harrison WR, Francomano CA, Prange CK, Lennon GG, Deere M, Lawler J. Mutations in exon 17B of cartilage oligometric matrix protein (COMP) cause pseudoachondroplasia. Nat Genet 1995;10:325-329.

Henry I, Bernheim A, Bernard M, Rest M van der, Kimura T, Jeanpierre C, Barichard F, Berger R, Olsen BR, Ramirez F, Junien C. Mapping of a human fibrillar collagen gene, proα1(XI) (COL11A1), to the p21 region of chromosome 1. Genomics 1988;3:87-90.

Hermodsson I. Hereditary multiple epiphyseal disturbances. Acta Orthop Scand 1944;15:182-205.

Herring JA. Rapidly progressive scoliosis in multiple epiphyseal dysplasia. A case report. J Bone Joint Surg 1976;58A:703-704.

Herring JA, Hotchkiss BL. Legg-Pertes disease versus multiple epiphyseal dysplasia. Instructional case. J Pediatr Orthop 1987;7:341-343.

Hirsch IS. Generalized osteochondrodystrophy. J Bone Joint Surg 1937;19:297-313.

Hobaek A. Problems of hereditary chondrodysplasias. 1961. Oslo University Press, Oslo.

Hodkinson HM. Double patellae in multiple epiphyseal dysplasia. J Bone Joint Surg 1962;44B:569-572.

Hoefnagel D, Sycamore LK, Russel SW, Bucknall WE. Hereditary multiple epiphysial dysplasia. Ann Hum Genet 1967;30:201-210.

Horton WA. Molecular genetics of the human chondrodysplasias-1995. Eur J Hum Genet 1995;3:357-373.

Hulvey JT, Keats T. Multiple epiphyseal dyplasia. A contribution to the problem of spinal involvement. Amer J Roentgenol 1969;106:170-177.

Hunt DD, Ponseti IV, Pedrini-Mille A, Pedrini V. Multiple epiphyseal dysplasia in two siblings. Histological and biochemical analyses of epiphyseal plate cartilage in one. J Bone Joint Surg 1967;49A:1611-1627.

Hunter AGW, MacDonald I. Mild multiple epiphyseal dysplasia with subtle dysmorphic features and joint limitation. Dysmorphol Clin Genet 1989;3:8-12.

Hurley LS, Asling CW. Localized epiphyseal dysplasia in offspring of manganese-deficient rats. Anat Rec 1963;145:25-37.

Ikegawa S, Nagano A, Nakamura K. A case of multiple epiphyseal dysplasia complicated by unilateral Perthes' disease. Acta Orthop Scand 1991;62:606-608.

Ingram RR. The shoulder in multiple epiphyseal dysplasia. J Bone Joint Surg 1991; 73B:277-279.

Ingram RR. Early diagnosis of multiple epiphyseal dysplasia. J Pediatr Orthop 1992; 12:241-244.

Jackson WPU, Hanelin J, Albright F. Metaphyseal dysplasia, epiphyseal dysplasia, diaphyseal dysplasia, and related conditions. II: Multiple epiphyseal dysplasia; its relation to other disorders of epiphyseal development. Arch Intern Med 1954; 94:886-901.

Jacobs P. Multiple epiphyseal dysplasia. Progr Pediatr Radiol 1973;4:309-324. (Intrinsic diseases of bones. Ed: HJ Kaufmann, Karger, Basel).

Jacobs PA. Dysplasia epiphysialis multiplex. Clin Orthop 1968;58:117-128.

Jansen M. Über atypische Chondrodystrophie (Achondroplasie) und über eine noch nicht beschriebene angeborene Wachstumsstörung des Knochensytems: Metaphysäre Dysostosis. Zeitschr Orthop Chir 1934;61:253-286.

Juberg RC, Holt JF. Inheritance of multiple epiphyseal dysplasia, tarda. Am J Hum Genet 1968;20:549-563.

Juberg RC. Heredity and multiple epiphyseal dysplasia. J Amer Med Ass 1977;237: 2600.

Katzenstein PL, Malemud CJ, Pathria MN, Carter JR, Sheon RP, Moskowitz RW. Early-onset primary osteoarthritis and mild chondrodysplasia. Radiographic and pathologic studies with an analysis of cartilage proteoglycans. Arthritis Rheum 1990;33:674-684.

Kaufman EE, Coventry MB. Multiple epiphysial dysplasia in a mother and son. Staff Meet Mayo Clin 1963;38:115-124.

Kaufmann HJ. Classification of the skeletal dysplasias and the radiologic approach to their differentiation. Clin Orthop 1976;114:12-17.

Keene DR, Oxford JT, Morris NP. Ultrastructural localization of collagen types II, IX, and XI in the growth plate of human rib and fetal bovine epiphyseal cartilage:

type XI collagen is restricted to thin fibrils. J Histochem Cytochem 1995;43:967-979.

Khermosh O, Wientroub S. Dysplasia epiphysealis capitis femoris. Meyer's dysplasia. J Bone Joint Surg 1991;73B:621-625.

Kingsley DM, Bland AE, Grubber JM, Marker PC, Russell LB, Copeland NG, Jenkins NA. The mouse short ear skeletal morphogenesis locus is associated with defects in a bone morphogenetic member of the TGFβ superfamily. Cell 1992;71:399-410.

Knowlton RG, Weaver EJ, Struyk AF, Knobloch WH, King RA, Norris K, Shamban A, Uitto J, Jimenez SA, Prockop DJ. Genetic linkage analysis of hereditary arthro-ophthalmopathy (Stickler syndrome) and the type II procollagen gene. Am J Hum Genet 1989;45:681-688.

Knowlton RG, Cekleniak JA, Cohn DH, Briggs MD, Hoffman SMG, Brandriff BF, Olsen AS. High-resolution genetic and physical mapping of multiple epiphyseal dysplasia and pseudoachondroplasia mutations at chromosome 19p13.1-p12. Genomics 1995;28:513-519.

Koppers B. Dysplasia epiphysealis multiplex-szintigraphische, röntgenologische und klinische Korrelation. Fortschr Röntgenstr 1982;137:291-305.

Kozlowski K, Bartkowiak K. Dysplasia epiphysealis multiplex. Report of a case. Radiol Diagn 1965;6:361-367.

Kozlowski K, Budzinska A. Combined metaphyseal and epiphyseal dysostosis. Report of two cases-one, in which metaphyseal changes predominate, and a second one, in which epiphyseal changes are more marked. Am J Roentgenol 1966;97:21-30.

Kozlowski K, Lipska E. Hereditary dysplasia epiphysealis multiplex. Clin Radiol 1967;18:330-336.

Kuivaniemi H, Tromp G, Prockop DJ. Mutations in collagen genes: causes of rare and some common diseases in humans. FASEB J 1991;5:2052-2060.

Lachman RS, Rimoin DL, Hollister DW. Arthrography of the hip. A clue to the pathogenesis of the epiphyseal dysplasias. Radiology 1973;108:317-322.

Lachman RS, Rimoin DL, Hollister DW. Hip arthrography in the epiphyseal dysplasias. Birth Defects 1974;10:186-195.

Lane JM, Weiss C. Review of articular cartilage collagen research. Arthritis Rheum 1975;18:553-562.

Langer LO, Schaefer GB, Wadsworth DT. Patient with double heterozygosity for achondroplasia and pseudoachondroplasia, with comments on these conditions

and the relationship between pseudoachondroplasia and multiple epiphyseal dysplasia, Fairbank type. Am J Med Genet 1993;47:772-781.

Lathrop GM, Lalouel JM, Julier C, Ott J. Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. Am J Hum Genet 1985;37: 482-498.

Laurencin CT, Ferriter PJ, Millis MB. Oblique proximal tibial osteotomy for the correction of tibia vara in the young. Clin Orthop 1996;327:218-224.

Leeds NE. Epiphysial dysplasia multiplex. Amer J Roentgenol 1960;84:506-510.

Lehman WL, Murray DM. Multiple epiphyseal dysplasia: A longitudinal family study. Orthopedics 1981;4:38-46.

Levy W, Mazumdar H, Morales F. Dysplasia epiphysialis multiplex. J Pediatr 1957;50:724-729.

Lie SO, Siggers DC, Dorst JP, Kopits SE. Unusual multiple epiphyseal dysplasias. Birth defects 1974;10:165-185.

Litchman HM, Chirls M. Dysplasia epiphysalis multiplex. Bull Hosp Joint Dis 1958;19:88-99.

Lovell WW, Winter RB. Pediatric orthopaedics, 2nd edition. Philadelphia: JB Lippincott Company, 1986.

Lowry RB, Wood BJ. Syndrome of epiphyseal dysplasia, short stature, microcephaly and nystagmus. Clin Genet 1975;8:269-272.

Lowry RB, Wood BJ. Multiple epiphyseal dysplasia and Turner syndrome variant (45, XO/46,X Ring) associated with advanced maternal age. Birth Defects 1977;13:211-220.

Lowry RB, Wood BJ, Cox TA, Hayden MR. Epiphyseal dysplasia, microcephaly, nystagmus, and retinitis pigmentosa. Am J Med Genet 1989;33:341-345.

Lowry RB, Wesenberg RL, Hall JG. Syndrome of multiple epiphyseal dysplasia (Ribbing type) with rhizomelic shortness, cleft palate, and micrognathia in two unrelated patients. Am J Med Genet 1996;63:55-61.

MacDermot KD, Roth SC, Hall C, Winter RM. Epiphyseal dysplasia of the femoral head, mild vertebral abnormality, myopia, and sensorineural deafness: report of a pedigree with autosomal dominant inheritance. J Med Genet 1987;24:602-608.

MacKenzie WG, Bassett GS, Mandell GA, Scott CI. Avascular necrosis of the hip in multiple epiphyseal dysplasia. J Pediatr Orthop 1989;9:666-671.

Mandell GA, MacKenzie WG, Scott CI, Harcke HT, Wills JS, Basset GS. Identification of avascular necrosis in the dysplastic proximal femoral epiphysis. Skeletal Radiol 1989;18:273-281.

Mansoor IA. Dysplasia epiphysealis multiplex. Clin Orthop 1970;72:287-292.

Maroteaux P. Epiphyseal dysplasia, multiple. In: Bergsma DR. Birth Defects Compendium. New York, National Foundation, March of Dimes: Alan R Liss Inc 1979: 409.

Mau H, Schmidt HW. Der konstitutionell-dysostotische Perthes und die Skelettreifungshemmungen beim eigentlichen Perthes. Z Orthop 1960;93:515-530.

Maudsley RH. Dysplasia epiphysialis multiplex. A report of fourteen cases in three families. J Bone Joint Surg 1955;37B:228-240.

Maynard JA, Cooper RR, Ponseti IV. A unique rough surfaced endoplasmic reticulum inclusion in pseudoachondroplasia. Lab Invest 1972;26:40-44.

Mayne R, Irwin MH. Collagen types in cartilage. In: Kuettner KE, Schleyerbach R, Hascall VC. Articular cartilage biochemistry. New York: Raven Press 1986:23-38.

McKusick VA, Francomano CA, Antonarakis SE. Mendelian inheritance in man: a catalog of human genes and genetic disorders, 11th edition. Baltimore: John Hopkins University Press 1994.

Mena HR, Pearson EO. Multiple epiphyseal dysplasia. A family case report. JAMA 1976;236:2629-2633.

Mendler M, Eich-Bender SG, Vaughan L, Winterhalter KH, Bruckner P. Cartilage contains mixed fibrils of collagen types II, IX and XI. J Cell Biol 1989;108:191-197.

Meyer J. Dysplasia epiphysealis capitis femoris. A clinical-radiological syndrome and its relationship to Legg-Calvé-Pertes disease. Acta Orthop Scand 1964;34:183-197.

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acids Res 1988;16:1215.

Molay M, Wilson R, Lasker A. Os trigonum syndrome in a patient with multiple epiphyseal dysplasia. J Foot Surg 1982;21:265-268.

Monty CP. Familial Perthes' disease resembling multiple epiphysial dysplasia. J Bone Joint Surg 1962;44B:565-568.

Mourik JBA van, Schaap C, Nollen AJG. Een Nederlandse familie met erfelijke gewrichtsklachten; multipele epifysaire dysplasie. Ned Tijdschr Geneeskd 1993; 137:32-36.

Müller W. Das Bild der multiplen erblichen Störung der Epiphysenverknöcherung. Zeitschr f Orthop 1939A;69:257-282.

Müller W. Die erbliche multiple Störung der Epiphysenverknöcherung als typisches Krankheitsbild. Fortschr Röntgenstr 1939B;59:65-69.

Müller-Glauser W, Humbel B, Glatt M, Sträuli P, Winterhalter KH, Bruckner P. On the role of type IX collagen in the extracellular matrix of cartilage: type IX collagen is localized to intersections of collagen fibrils. J Cell Biol 1986;102:1931-1939.

Munk J, Boldo P. Dysplasia epiphysealis multiplex: a case report. Israel M J 1961;20: 160-164.

Murphy MC, Shine IB, Stevens DB. Multiple epiphyseal dysplasia. Report of a pedigree. J Bone Joint Surg 1973;55A:814-820.

Nagamine M, Ohkuma S. Serum alkaline phosphatase isoenzymes linked to immunoglobulin G. Clin Chim Acta 1975;65:39-46.

Nakata K, Ono K, Miyazaki JI, Olsen BR, Muragaki Y, Adachi E, Yamamura KI, Kimura T. Osteoarthritis associated with mild chondrodysplasia in transgenic mice expressing α1(IX) collagen chains with a central deletion. Proc Natl Acad Sci USA 1993;90:2870-2874.

Nau MM, Brooks BJ, Battey J, Sausville E, Gazdar AF, Kirsch IR, McBride OW, Bertness V, Hollis GF, Minna JD. L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. Nature 1985;318:69-73.

Nevin NC, Thomas PS, Hutchinson J. Syndrome of short stature, microcephaly, mental retardation, and multiple epiphyseal dysplasia-Lowry-Wood syndrome. Am J Med Genet 1986;24:33-39.

Ödman P. Hereditary enchondral dysostosis. Acta Radiol 1959;52:97-113.

Oehlmann R, Summerville GP, Yeh G, Weaver EJ, Jimenez SA, Knowlton RG. Genetic linkage mapping of multiple epiphyseal dysplasia to the pericentromeric region of chromosome 19. Am J Hum Genet 1994;54:3-10.

Olsen BR. The next frontier: molecular biology of extracellular matrix. Connect Tissue Res 1989;23:115-121.

Op de Beeck K, Vandenbosch G, Lateur L, Baert AL. Dysplasia epiphysealis hemimelica (Trevor's disease). J Belge Radiol 1993;76:386-387.

Patrone NA, Kredich DW. Arthritis in children with multiple epiphyseal dysplasia. J Rheumatol 1985;12:145-149.

Perälä M, Hänninen M, Hästbacka J, Elima K, Vuorio E. Molecular cloning of the human α2(IX) collagen cDNA and assignment of the human COL9A2 gene to chromosome 1. FEBS Lett 1993;319:177-180.

Pfeiffer RA, Jünemann G, Polster J, Bauer H. Epiphyseal dysplasia of the femoral head, severe myopia and perceptive hearing loss in three brothers. Clin Genet 1973;4:141-144.

Poole AR, Matsui Y, Hinek A, Lee ER. Cartilage macromolecules and the calcification of cartilage matrix. Anat Rec 1989;224:167-179.

Poole CA, Flint MH, Beaumont BW. Morphology of the pericellular capsule in articular cartilage revealed by hyaluronidase digestion. J Ultrastruct Res 1985;91: 13-23.

Poznanski AK, Garn SM, Nagy JM, Gall JC. Metacarpophalangeal pattern profiles in the evaluation of skeletal malformations. Radiology 1972;104:1-11.

Poznanski AK. Pattern profile comparisons: differences and similarities. Ann Radiol 1984;27:89-96.

Pronk JC, Beemer FA, Geraedts JPM, Leschot NJ, Niermeijer MF. Medische genetica, vijfde geheel herziene druk. Utrecht: Wetenschappelijke uitgeverij Bunge, 1994.

Rasmussen PG, Reimann I. Multiple epiphyseal dysplasia, with special reference to histological findings. Acta Pathol Microbiol Scand 1973;81:381-389.

Rasmussen PG. Multiple epiphyseal dysplasia. 2. Morphological and histochemical investigation of cartilage matrix, particularly in the pre-calcification stage. Acta Path Microbiol Scand 1975;83:493-502.

Reginato AM, Sanz-Rodriguez C, Jimenez SA. Biosynthesis and characterization of type X collagen in human fetal epiphyseal growth plate cartilage. Osteoarthritis & Cartilage 1995;3:105-116.

Rennell C, Steinbach HL. Epiphyseal dysostosis without dwarfism. Am J Roentgenol 1970;108:481-487.

Resnick D. Diagnosis of bone and joint disorders, 3rd edition. Philadelphia: WB Saunders Company, 1995.

Rest M van der, Mayne R. Type IX collagen proteoglycan from cartilage is covalently cross-linked to type II collagen. J Biol Chem 1988;263:1615-1618.

Ribbing S. Studien über hereditäre multiple Epiphysenstörungen. Acta Radiol (Stockholm) 1937, Suppl 34:77-107.

Rimoin DL, Rasmussen IM, Briggs MD, Roughley PJ, Gruber HE, Warman ML, Olsen BR, Hsia YE, Yuen J, Reinker K, Garber AP, Grover J, Lachman RS, Cohn DH.

A large family with features of pseudoachondroplasia and multiple epiphyseal dysplasia: exclusion of seven candidate gene loci that encode proteins of the cartilage extracellular matrix. Hum Genet 1994;93:236-242.

Rubin P. Dynamic classification of bone dysplasias. Chicago: Year Book Medical Publishers, 1964.

Schlesinger AE, Poznanski AK, Pudlowski RM, Millar EA. Distal femoral epiphysis: normal standards for thickness and application to bone dysplasias. Radiology 1986;159:515-519.

Scott LG. Dysplasia epiphysealis multiplex. Proc Roy Soc Med 1952;45:452.

Sensenbrenner JA. Probable multiple epiphyseal dyslasia. Birth Defects 1974;10:419-421.

Shapiro F. Epiphyseal disorders. New England J Med 1987;317:1702-1710.

Shaw LM, Olsen BR. FACIT collagens: diverse molecular bridges in extracellular matrices. TIBS 1991;16:191-194.

Shephard E. Multiple epiphysial dysplasia. J Bone Joint Surg 1956;38B:458-467.

Short LA, Hume RL, Gudas CJ. Multiple epiphyseal dysplasia. A review of the literature and case report. J Am Podiatr Assoc 1979;69:695-699.

Silfverskiöld N. A 'forme fruste' of chondrodystrophia with changes simulating several of the known 'local malacias'. Acta Radiol 1925;4:44-57.

Silfverskiöld N. Sur la question de l'achondroplasie atypique et de sa forme périphérique. Acta Radiol 1926;5:223-235.

Silverman FN. Dysplasies épiphysaires: entité protéiforme. Ann Radiol 1961;4: 833-867.

Silverman FN. Multiple epiphyseal dysplasia. Caffey's Pediatric X-ray Diagnosis. Chicago, Yearbook Medical Publishers Inc. 1985;584-586.

Silverman FN. Reflections on epiphyseal dysplasias. AJR 1996;167:835-842.

Smith GN, Brandt KD. Hypothesis: can type IX collagen 'glue' together intersecting type II fibers in articular cartilage matrix? A proposed mechanism. J Rheumatol 1992;19:14-17.

Soodan VM, Mohan V, Padha SP. Multiple epiphyseal dysplasia. Indian J Pediatr 1978;45:132-136.

Spiers ASD, Meagher T, Ostlere SJ, Wilson DJ, Dodd CAF. Can MRI of the knee affect arthroscopic practice? J Bone Joint Surg 1993;75B:49-52.

Spranger J. The epiphyseal dysplasias. Clin Orthop 1976;114:46-60.

Stanescu R. Ultrastructural abnormalities of the chondrocytes of the growth cartilage of a type of poly-epiphyseal dysplasia with a probable autosomal recessive transmission. Biomedicine 1975;23:144-148.

Stanescu V, Maroteaux P, Stanescu R. The biochemical defect of pseudoachondroplasia. Eur J Pediatr 1982;138:221-225.

Stanescu R, Stanescu V, Muriel MP, Maroteaux P. Multiple epiphyseal dysplasia, Fairbank type: morphologic and biochemical study of cartilage. Am J Med Genet 1993;45:501-507.

Stösz H, Pesch H-J, Pontz B, Otten A, Spranger J. Wolcott-Rallison syndrome: diabetes mellitus and spondyloepiphyseal dysplasia. Eur J Pediatr 1982;138:120-129.

Tachdjian MO. Pediatric orthopaedics, 2nd edition. Philadelphia: WB Saunders Company, 1990.

Thompson MW, McInnes RR, Willard HF. Thompson & Thompson: Genetics in medicine, 5th edition. Philadelphia: WB Saunders Company 1991.

Tilstra DJ, Beyers PH. Molecular basis of hereditary disorders of connective tissue. Annu Rev Med 1994;45:149-163.

Toby EB, Koman LA, Bechtold RE. Magnetic resonance imaging of pediatric hip disease. J Pediatr Orthop 1985;5:665-671.

Treble NJ, Jensen FO, Bankier A, Rogers JG, Cole WG. Development of the hip in multiple epiphyseal dysplasia. Natural history and susceptibility to premature osteoarthritis. J Bone Joint Surg 1990;72B:1061-1064.

Vaughan L, Mendler M, Huber S, Bruckner P, Winterhalter KH, Irwin MI, Mayne R. D-periodic distribution of collagen type IX along cartilage fibrils. J Cell Biol 1988;106:991-997.

Versteylen RJ, Zwemmer A, Lorié CAM, Schuur KH. Multiple epiphyseal dysplasia complicated by severe osteochondritis dissecans of the knee. Incidence in two families. Skeletal Radiol 1988;17:407-412.

Vikkula M, Metsäranta M, Ala-Kokko L. Type II collagen mutations in rare and common cartilage diseases. Ann Med 1994;26:107-114.

Vikkula M, Mariman ECM, Lui VCH, Zhidkova NI, Tiller GE, Goldring MB, Beersum van SEC, Waal Malefijt de MC, Hoogen van de FHJ, Ropers HH, Mayne R, Cheah KSE, Olsen BR, Warman ML, Brunner HG. Autosomal dominant and recessive osteochondrodysplasias associated with the COL11A2 locus. Cell 1995; 80:431-437.

Villarreal T, Carnevale A, Mayén DG, Takenaga R, del Castillo V. Anthropometric studies in five children and their mother with a severe form multiple epiphyseal dysplasia. Am J Med Genet 1992;42:415-419.

Vizkelety T, Szijj E. Familiäres Auftreten der Dysostosis enchondralis. Z Orthop 1964:99:133-146.

Walker BA. Juvenile cataracts and multiple epiphyseal dysplasia in three sisters. Birth Defects 1969;5:315-317.

Wardale RJ, Duance VC. Quantification and immunolocalisation of porcine articular and growth plate cartilage collagens. J Cell Sci 1993;105:975-984.

Warman ML, Tiller GE, Polumbo PA, Seldin MF, Rochelle JM, Knoll JHM, Cheng SD Olsen BR. Physical and linkage mapping of the human and murine genes for the α1 chain of type IX collagen (COL9A1). Genomics 1993;17:694-698.

Warman ML, McCarthy MT, Perälä M, Vuorio E, Knoll JHM, McDaniels CN, Mayne R, Beier DR, Olsen BR. The genes encoding α2(IX) collagen (COL9A2) map to human chromosome 1p32.3-p33 and mouse chromosome 4. Genomics 1994;23:158-162.

Watt JK. Multiple epiphysial dysplasia. A report of four cases. Brit J Surg 1952;39: 533-535.

Waugh W. Dysplasia epiphysialis multiplex in three sisters. J Bone Joint Surg 1952;34B:82-87.

Weaver EJ, Summerville GP, Yeh G, Hervada-Page M, Oehlmann R, Rothman R, Jimenez SA, Knowlton RG. Exclusion of type II and type VI procollagen gene mutations in a five-generation family with multiple epiphyseal dysplasia. Am J Med Genet 1993;45:345-352.

Weinberg H, Frankel M, Makin M, Vas E. Familial epiphysial dysplasia of the lower limbs. J Bone Joint Surg 1960;42B:313-332.

Wenger DR, Ezaki M. Bilateral femoral head collapse in an adolescent with brachydactyly (multiple epiphyseal dysplasia tarda type 1c). J Pediatr Orthop 1981;1:267-271.

Wiles P. Multiple epiphyseal dysplasia. Proc R Soc Med 1938;32:279-281.

Wolcott CD, Rallison ML. Infancy-onset diabetes mellitus and multiple epiphyseal dysplasia. J Pediatr 1972;80:292-297.

Wordsworth P, Ogilvie D, Priestley L, Smith R, Wynne-Davies R, Sykes B. Structural and segregation analysis of the type II collagen gene (COL2A1) in some heritable chondrodysplasias. J Med Genet 1988;25:521-527.

Wotton SF, Duance VC, Fryer PR. Type IX collagen: a possible function in articular cartilage. FEBS 1988;234:79-82.

Wu JJ, Woods PE, Eyre DR. Identification of cross-linking sites in bovine cartilage type IX collagen reveals an antiparallel type II-type IX molecular relationship and type IX to type IX bonding. J Biol Chem 1992;267:23007-23014.

Wynne-Davies R, Fairbank TJ. Fairbank's atlas of general affections of the skeleton, 2nd edition. Edinburgh: Churchill-Livingstone, 1976.

Wynne-Davies R, Hall C. Two clinical variants of spondylo-epiphysial dysplasia congenita. J Bone Joint Surg 1982;64B:435-441.

Wynne-Davies R, Hall C, Ansell B. Spondylo-epiphysial dysplasia tarda with progressive arthropathy. A 'new' disorder of autosomal recessive inheritance. J Bone Joint Surg 1982;64B:442-445.

Wynne-Davies R, Gormley J. The prevalence of skeletal dysplasias. An estimate of their minimum frequency and the number of patients requiring orthopaedic care. J Bone Joint Surg 1985;67B:133-137.

Yamamoto T, Tohyama J, Koeda T, Maegaki Y, Takahashi Y. Multiple epiphyseal dysplasia with small head, congenital nystagmus, hypoplasia of corpus callosum, and leukonychia totalis: a variant of Lowry-Wood syndrome? Am J Med Genet 1995;56:6-9.

Yarrow DE. Multiple epiphyseal dysplasia. Proc R Soc Med 1938;32:315-316.

Yochum TR, Sprowl CG, Barry MS. Double patella syndrome with a form of multiple epiphyseal dysplasia. J Manipulative Physiol Ther 1995;18:407-410.

Dankwoord

In juni 1995 ontstond het idee om de studies, die tot dat moment verricht waren naar aanleiding van de familie met MED, uit te breiden, zodat een promotie mogelijk zou worden. Initiator van die studies was dr. Ad Nollen. Destijds, in 1991, hebben we ons niet gerealiseerd, dat het stamboomonderzoek tot de ontdekkingen zou leiden, die in dit proefschrift zijn beschreven.
Tijdens een telefoongesprek van een half uur met prof.dr. J.A.N. Verhaar ontstond het raamwerk voor dit proefschrift; de verdere gang van zaken is eigenlijk even vlot verlopen. Jan, ik ben je zeer veel dank verschuldigd voor de steeds stimulerende wijze waarop jij mij hebt begeleid. Met zeer veel energie heb je je ook ingelezen en ingewerkt in de genetische problematiek, die voor orthopeden (nog) geen alledaagse kost is.
Een groot gedeelte van dit proefschrift behandelt de genetische kant van MED. Beste dr. Edwin Mariman en drs. Ben Hamel, zonder jullie geduld tijdens de vele middagen, die we aan dit proefschrift hebben besteed, was het mij niet gelukt. Hetzelfde geldt voor de elektronenmicroscopie: dank, dr. Pieter Buma, voor je uitleg.
Een woord van dank ook aan de andere collegae, met wie ik de publicaties, die de basis vormden voor dit proefschrift, heb mogen maken: dr. Ad Nollen, drs. Cees Schaap, drs. Hans Weerdenburg, dr. Ed Lommen, drs. Herman Peters, dr. Bill Wilcox en de groep van prof. Bjorn Olsen; dr. Roel Kuyer dank ik voor zijn adviezen aangaande kraakbeenonderzoek, dr. Fedde van der Graaf voor zijn statistische ondersteuning.
Prof.dr. M.F. Niermeijer ben ik veel dank verschuldigd voor de zeer waardevolle correcties in de laatste fase. Ook dank ik de overige leden van de beoordelingscommissie, prof.dr. S.L.S. Drop en prof.dr. M. Meradji, voor hun beoordeling van het manuscript.
Promoveren doe je niet alleen. Zeker waar het de gegevens voor de stamboomonderzoeken betreft heb ik veel hulp gehad van onze secretaresses Karin van Hoof, Josée Kraker, Natascha Sepers, Ingrid de Wert, Sylvia Christiaans en Anita van Herwijnen. Literatuur werd steeds maar weer opgevraagd door Marion Heymans en Henriëtte van de Griendt. Foto's, tekeningen en posters werden op vakkundige wijze verzorgd door Henk Dinnissen en Hanneke Keijers. Nigel Martin gaf adviezen naar aanleiding van mijn eerste Engelse brieven, drs. Anne Myrthe Iken corrigeerde alle manuscripten. De computer werd aan de praat gehouden door Ed Smeets; Pieter van Gerwen tekende de stambomen. Mijn nog niet genoemde collega's Arthur Lim, Harm Sala en Paul Winkelman en onze assistenten in opleiding dank ik voor hun belangstelling aangaande mijn vorderingen.
Mijn oprechte dank gaat uit naar de in mijn proefschrift beschreven patiënten met MED en hun familieleden. Wanneer extra onderzoek was gewenst, bijvoorbeeld bloedafname of röntgenonderzoek, was een enkel telefoontje vaak voldoende. Ik hoop

werkelijk, dat de uitkomsten van deze dissertatie in de toekomst zullen leiden tot meer therapeutische mogelijkheden voor MED.
Grote dank ben ik verschuldigd aan hen die verantwoordelijk waren voor mijn opleiding: drs. A. Axler, die mijn interesse heeft gewekt voor de orthopedie, dr. T.I. Yo, mijn opleider algemene heelkunde, en prof.dr. A.J. van der Linden, mijn opleider orthopedie. Verder ben ik er trots op, dat Mart Bender en Frank Faber naast mij willen staan bij de verdediging van het proefschrift.

En dan natuurlijk het thuisfront. Beste pa en ma, dank jullie wel voor de uitstekende basis die ik heb gehad en jullie niet aflatende interesse. Beste schoonouders, jullie bedank ik ook voor jullie stimulerende belangstelling aangaande mijn vorderingen. Lieve Elly, zonder jouw steun en liefde was dit boekje niet mogelijk geweest. Jij weet in ons huis altijd de sfeer zo te houden, dat het steeds leuk bleef om nog 'even naar boven te gaan'. Zo heb ik het promoveren altijd als zeer plezierig ervaren. En ten slotte, lieve Casper en Thijs: het boekje is klaar; we gaan een feestje bouwen!

Curriculum vitae

Jan van Mourik werd op 4 maart 1957 geboren in Gorinchem. Na de lagere school en het gymnasium Camphusianum te Gorinchem werd de medische studie aangevangen aan de Erasmus Universiteit te Rotterdam, hetgeen resulteerde in het artsdiploma in 1982.

Na een jaar orthopedie in het Sint Clara Ziekenhuis te Rotterdam (drs. A. Axler) en veertien maanden militaire dienst in de Generaal Winkelman kazerne te Nunspeet, werd de vooropleiding algemene heelkunde gevolgd in het Sint Clara Ziekenhuis te Rotterdam (dr. T.I. Yo). De opleiding tot orthopedisch chirurg vond plaats in het Academisch Ziekenhuis Maastricht (prof.dr. A.J. van der Linden).

Na registratie op 1 april 1992 volgde associatie met dr. T.E. Lim, dr. A.J.G. Nollen, drs. H.A.G.M. Sala en dr. P.A.M. Winkelman in het Sint Joseph Ziekenhuis te Veldhoven. Van Mourik is eveneens lid van het samenwerkingsverband traumatologie, waarin alle algemeen chirurgen en orthopeden van het Sint Joseph Ziekenhuis participeren.

Colofon

Omslagontwerp	: Nick Seijkens, Eersel
Vormgeving binnenwerk	: Thea de Beer, Leiden
Illustraties en röntgenfoto's	: Henk Dinnissen en Hanneke Keijers, Veldhoven
Correctie Engels	: Anne-Myrthe Iken, Valkenswaard
Drukwerk	: Elinkwijk bv, Utrecht

MULTIPLE EPIPHYSEAL DYSPLASIA

A Clinical and Molecular Genetic Study