DEFECTS IN THE GENES CODING FOR CARTILAGE EXTRACELLULAR MATRIX PROTEINS AS A CAUSE OF OSTEOARTHRITIS AND MULTIPLE EPIPHYSEAL DYSPLASIA

EVELIINA JAKKULA

Faculty of Medicine,
Department of Medical Biochemistry
and Molecular Biology,
Biocenter Oulu,
University of Oulu

OULU 2005

EVELIINA JAKKULA

DEFECTS IN THE GENES CODING FOR CARTILAGE EXTRACELLULAR MATRIX PROTEINS AS A CAUSE OF OSTEOARTHRITIS AND MULTIPLE EPIPHYSEAL DYSPLASIA

Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in Auditorium of the Medipolis (Kiviharjuntie 11), on May 27th, 2005, at 10 a.m.

OULUN YLIOPISTO, OULU 2005

Copyright © 2005
University of Oulu, 2005

Supervised by
Professor Leena Ala-Kokko

Reviewed by
Docent Kati Elima
Docent Marjo Kestilä

ISBN 951-42-7732-5 (nid.)
ISBN 951-42-7733-3 (PDF) http://herkules.oulu.fi/isbn9514277333/

ISSN 0355-3221 http://herkules.oulu.fi/issn03553221/

OULU UNIVERSITY PRESS
OULU 2005

Jakkula, Eveliina, Defects in the genes coding for cartilage extracellular matrix proteins as a cause of osteoarthritis and multiple epiphyseal dysplasia

Faculty of Medicine, Department of Medical Biochemistry and Molecular Biology, Biocenter Oulu, University of Oulu, P.O.Box 5000, FIN-90014 University of Oulu, Finland

2005

Oulu, Finland

Abstract

The role of sequence variations in genes encoding cartilage extracellular matrix (ECM) proteins were studied in osteoarthritis (OA) and multiple epiphyseal dysplasia (MED). The cartilage collagen genes *COL2A1*, *COL9A1*, *COL9A2*, *COL9A3*, *COL11A1*, and *COL11A2* were screened for sequence variations in 72 Finnish probands and one US family with primary early-onset hip and/or knee OA. Altogether 239 sequence variations were found, of which 16 were not present in the controls. Seven of the unique variations — four in *COL11A1*, two in *COL11A2*, and one in *COL2A1* — were studied further, because they resulted in the substitution of conserved amino acids or were predicted to affect mRNA splicing. Association analysis was performed by genotyping 6–12 common polymorphisms from each gene in 72 OA patients and 103 controls; no common predisposing alleles were identified. The results, however, suggest that mutations in the minor cartilage collagen genes can be the cause of OA in a subgroup of OA patients.

Two MED families with clinical and radiographic features suggestive of a collagen IX mutation were studied. Mutation screening of *COL9A1*, *COL9A2*, and *COL9A3* yielded negative results. Instead, an R718W mutation in *COMP* was identified in both families. Clinical and radiographic overlap between patients with collagen IX mutations and patients with *COMP* mutations points to a common supramolecular complex pathogenesis.

Clinical, radiological and molecular analyses of known MED genes were performed on a cohort of 29 consecutive MED patients. The *DTDST* mutation was identified in four patients (14%), the *COMP* mutation in three (10%), and the *MATN3* mutation in three (10%). Two new distinct phenotypic entities were identified in patients in whom no mutation was found. The findings suggest that mutations in the above mentioned known MED genes are not the major cause of MED and are responsible for less than half of the cases. The existence of additional MED loci is supported by the exclusion of known loci and finding of the specific subgroups among these patients.

The results suggest that genetic defects in ECM genes can predispose to OA and cause MED, even though the major genes involved in both disorders remain to be found.

Keywords: chondrodysplasia, multiple epiphyseal dysplasia, osteoarthritis

Acknowledgements

This work was carried out at the Collagen Research Unit of the Department of Medical Biochemistry and Molecular Biology, University of Oulu, and Biocenter Oulu, and at the Center for Gene Therapy, Tulane University Health Sciences Center, New Orleans, USA, during the years 1999-2005.

I wish to express my deepest gratitude to my supervisor, Professor Leena Ala-Kokko, for her continuous encouragement and never-ending optimism throughout these years and for arranging me the opportunity for me to work at the Center for Gene Therapy in New Orleans. I would like to express my sincere thanks to Professors Peppi Karppinen, Kari Kivirikko, and Taina Pihlajaniemi for providing excellent research facilities and maintaining a high-standard scientific environment. In addition, I wish to express my respect for Professor Ilmo Hassinen for his outstanding scientific work in the field of energy metabolism. My sincere thanks are to Professor Darwin J Prockop for giving me the honour of working in his laboratory in New Orleans.

Docents Kati Elima and Marjo Kestilä are acknowledged for their thorough and valuable comments on this thesis. I wish to thank Sandra Hänninen, M.Sc., for her careful revision of the language of the manuscript.

All the collaborators who participated in the projects presented in this thesis deserve my sincere thanks. I am grateful to Miia Melkoniemi for teaching me the basics of lab work at start of this research project and for fruitful collaboration with the osteoarthritis project throughout these years. I had the opportunity to work alongside a team of expert clinicians, including Professor Heikki Kröger, Docent. Ilkka Kiviranta, Professor Andrea Superti-Furga, and Docent Outi Mäkitie, and their clinical and scientific expertise has been essential in completing the research projects presented in this thesis. Harald H.H. Göring is acknowledged for his collaboration with the statistical aspects of the osteoarthritis project.

The colleagues at the Department of Medical Biochemistry and Molecular Biology, University of Oulu, and at the Center for Gene Therapy, New Orleans deserve my warmest thanks. I want to especially thank all the members of group Ala-Kokko, for the cheerful moments and for the scientific and non-scientific discussions in the offices and outside the lab. I wish to thank Minna Männikkö, our always friendly post-doc, for her help with research problems and for her support and friendship over the years. The time

spent in Philadelphia and New Orleans was made much more enjoyable by sharing the work and leisure time with Noora Noponen-Hietala, Minta Lumme, Jaana Peters, and Ahmad Sahraravand; thank you for all the cheerful memories.

I wish to thank Aira Harju, Minta Lumme, Justing Manges, Marjorie McGants, Helena Satulehto, Marika Oikarinen, and Christina Troxell for their excellent technical assistance.

I warmly thank our group members Heini Hartikka, Noora Noponen-Hietala and Miia Melkoniemi for their contributions to the practical arrangement of the dissertation. I thank them for sharing the sometimes hectic moments during the preparation of the doctoral dissertation and during the organization of the party.

My sincere thanks go to Auli Kinnunen and Marja-Leena Kivelä for their always efficient and friendly secretial assistance, to Pertti Vuokila, Marja-Leena Karjalainen, and Seppo Lähdesmäki for keeping the everyday lab-life running smoothly, and to our computer crew, especially to Risto Helminen, for their help with computers. The assistance of the staff of the Center for Gene Therapy, New Orleans, is also greatly acknowledged.

I wish to express my warmest gratitude to my parents, Eero and Maaret, for all their loving care and support over the years, and for giving me an excellent basis for my life. My sister Elina and brother Eerikki both deserve my sincere thanks for many joyful moments , which brightened the days filled with research work and medical studies, and for their friendship. I want to warmly thank my colleagues and dear friends, especially Outi Jauhola, Virpi Koskela, and Elise Saarela, for sharing the many aspects of being a medical student and the first steps of our clinical carrier, and for being such good friends, even if I sometimes seemed to be constantly in the lab.

This work was financially supported by the Finnish Medical Foundation and the Oulu University Scholarship Foundation.

Oulu, March 2005 Eveliina Jakkula

Abbreviations

ACG1B	achondrogenesis type 1B
AO2	atelosteogenesis type 2
BMI	body mass index
BMP	bone morphogenetic protein
bp	base pair(s)
C-	carboxy-
cDNA	complementary DNA
CDMP1	cartilage-derived morphogenetic protein 1
CI	confidence interval
COL	collagenous domain
COLyAx	human gene for the α(x) chain of collagen y
COMP	cartilage oligomeric matrix protein
CSGE	conformation-sensitive gel electrophoresis
DTD	diastrophic dysplasia
DTDST	diastrophic dysplasia sulphate transporter
EBV	Ebstein-Barr virus
ECM	extracellular matrix
EGF	epidermal growth factor
FGF	fibroblast growth factor
FRZB	frizzled motif associated with bone development
Gly	glycine
IGF-1	insulin like growth factor-1
Ihh	Indian hedgehog protein
IL-1	interleukin-1
IL4R	interleukin 4 receptor α
JSN	joint space narrowing
kb	kilobasepair(s)
MATN3	matrilin-3
MED	multiple epiphyseal dysplasia
MLS	multipoint lod score
MMP	matrix metalloproteinase

MRI	magnetic resonance imaging
mRNA	messanger RNA
N-	amino-
NC	non-collagenous
OA	osteoarthritis
OPLL	ossification of the posterior longitudinal ligament of the spine
OR	odds ratio
OSMED	otospondylomegaepiphyseal dysplasia
PCR	polymerase chain reaction
PSACH	pseudoachondroplasia
PTHrP	parathyroid hormone related protein
rER	rough endoplasmic reticulum
rMED	recessive multiple epiphyseal dysplasia
ROA	radiological OA
RT-PCR	reverse transcriptase PCR
SD	standard deviation
SED	spondyloepiphyseal dysplasia
SNP	single nucleotide polymorphism
T3-	type III repeat
TGF-β	transforming growth factor- β
THR	total hip replacement
TKR	total knee replacement
TNF-α	tumor necrosis factor- α
VDR	vitamin D receptor
VEGF	vascular endothelial growth factor
vWFA	vonWillebrand factor A-like domain
X	any amino acid
Y	any amino acid

List of original articles

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I Jakkula E, Melkoniemi M, Kiviranta I, Lohiniva J, Räinä SS, Warman ML, Ahonen K, Kröger H, Göring HHH, Ala-Kokko L (2005). The role of sequence variations within the genes encoding collagen II, IX and XI in Non-syndromic, early-onset osteoarthritis. Osteoarthritis Cartilage, in press

II Jakkula E, Lohiniva J, Capone A, Bonafe L, Marti M, Schuster V, Giedion A, Eich G, Boltshauser E, Ala-Kokko L, Superti-Furga A (2003). A recurrent R718W mutation in *COMP* results in multiple epiphyseal dysplasia with mild myopathy: clinical and pathogenetic overlap with collagen IX mutations. J Med Genet 40:942-948

III Jakkula E, Mäkitie O, Czarny-Ratajzak M, Jackson GC, Damignani R, Susic M, Briggs MD, Cole WC, Ala-Kokko L (2005). Mutations in the known genes are not the major cause for MED; distinctive phenotypic entities among patients with no identified mutations. Eur J Hum Genet 13:292-301

In addition, some unpublished data are presented.

Contents

1 Introduction

Hyaline cartilage is the most common, prototypic form of cartilage and it is found in the articular surfaces of joints, cartilaginous anlagen of developing bone, epiphyseal growth plates, costochondral cartilages, and the cartilaginous parts of the trachea, bronchi, larynx, and nose. Hyaline cartilage has an important role throughout the human's lifetime from the development of the cartilaginous anlagen of the skeleton during the embryonic stage until adulthood, when its main role is to enable smooth joint articulation that resists the tensile and compressive forces applied to the joints. The unique composition and structure of the hyaline cartilage makes these functions possible.

The mature hyaline cartilage contains only a few cells embedded in an abundant extracellular matrix (ECM). The major components of this ECM are collagens and proteoglycans. Collagen II is the major cartilage collagen interacting with the minor cartilage collagens IX and XI to form heterotypic collagen fibrils. Collagens III, VI, X, XII, XIII, XIV, and XVI also contribute to the mature cartilage matrix. The main proteoglycan of cartilage is aggrecan, which is mainly responsible for the compressive stiffness of the hyaline cartilage, whereas collagen fibrils are responsible for providing the tensile strength of the cartilage tissue. Noncollagenous proteins present in cartilage, such as cartilage oligomeric matrix protein (COMP) and matrilins, have an important function in cartilage structure by providing interactions between different components of the ECM as well as in cell-matrix interactions, and play a role in cartilage metabolism and regulating chondrocyte proliferation.

Defects in the genes encoding these components can lead to disturbed formation and maintenance of hyaline cartilage. This may cause a wide spectrum of disorders ranging from perinatally lethal, severe chondrodysplasias to mild chondrodysplasias, where precocious osteoarthritis as a result of the degeneration of articular cartilage is often part of the phenotype. One example of the mild chondrodysplasias is multiple epiphyseal dysplasia (MED), in which the normal endochondral ossification process is disturbed. Abnormalities in epiphyseal and growth plate cartilage result, leading to delayed and irregular ossification. Patients usually present symptoms such as joint pain, stiffness, or waddling gait during childhood or later in life, as early-onset osteoarthritis.

Osteoarthrosis or osteoarthritis (OA) is the most common joint disorder affecting millions of people worldwide. The hallmark finding of OA is the gradual degradation of

articular cartilage. The etiology of OA is still not well understood, but it has long been known to have a strong genetic component. Despite the numerous studies performed and advances in genetics, the major disease-causing or predisposing genes in OA have not yet been identified.

The characterization of genetic defects will not only give us more information about the function of the proteins, but will also lead to a better understanding of the pathogenesis of the diseases. With that knowledge, it might be possible in the future to develop treatments for e.g. OA patients which would not only treat the symptoms but also address the cause of the disease.

The goal of this thesis was to study the role of sequence variations in the cartilage collagen genes in primary hip and knee OA, as well as to study the role of mutations in known MED genes and the phenotype-genotype correlation in MED. Mutation and association analyses of *COL2A1*, *COL9A1*, *COL9A2*, *COL9A3*, *COL11A1,* and *COL11A2* of patients with primary, early-onset hip and/or knee OA were performed. The genotype-phenotype correlation in MED was further improved by a thorough mutation and clinical analysis of MED patients, resulting in the identification of two new phenotypic entities among patients in whom the mutation screening of known MED genes (*COMP*, *COL9A1*, *COL9A2*, *COL9A3*, *MATN3,* and *DTDST*) had yielded negative results.

2 Review of the literature

2.1 Cartilage

Cartilage is a specialized form of connective tissue, and there are three different types of cartilage in the human body. The most common type of cartilage is hyaline cartilage, which originally forms the cartilaginous model of the developing skeleton and is later replaced by bone during the process of endochondral ossification of the long bones and vertebrae. It can also be found in the growth plates until puberty. In adults, hyaline cartilage can be found in the articular surfaces of joints, in costochondral cartilages, and in the cartilaginous parts of the trachea, bronchi, larynx, and nose. The other types of cartilage are fibrocartilage, found in the annulus fibrosus of the intervertebral disk, tendinous and ligamentous insertions, menisci, the symphysis pubis, and insertions of joint capsules, and elastin cartilage, found in the pinna of the ears, in the epiglottis, and in the arytenoid cartilages of the larynx. (Schiller 1994.)

The properties of these different kinds of cartilage are due to their different structures. Two features common to all these cartilage types are the relatively low content of cells, chondrocytes, and the abundance of ECM secreted by the chondrocytes. The cartilage tissue is highly hydrated, and the main components of the ECM are collagens and proteoglycans, where collagens provide the fibril framework for the amorphous material consisting of mainly of proteoglycans and other proteins. The fibril network of hyaline cartilage consists mainly of collagen II fibrils, whereas in fibrocartilage the main fibril-forming collagen is collagen I. In elastin cartilage the main structural component is elastin. Therefore, the different functional properties of the cartilage types arise from their structural components. (Schiller 1994.)

2.2 Development of the skeleton and the formation of synovial joints

Skeletal development has fascinated quite a large number of researchers, and currently the major steps of skeletal development are relatively well known for the mouse; the

molecular background and regulation of these processes are still being studied in more detail. The main phases of skeletal development are summarized below. (For reviews see DeLise *et al.* 2000, Olsen *et al.* 2000 and Kronenberg 2003.)

The formation of the skeleton is a highly organized, complex process that begins with the migration of undifferentiated mesenchymal cells to areas destined to become bone and joints. The next step in the process is mesenchymal cell condensation. Cells from the cranial neural crest condense to form craniofacial skeleton, those from the somites give rise to the axial skeleton, and cells from the lateral mesodermal plate form condensations that give rise to the limb bones. From this point on, the development continues differently in the craniofacial versus other parts of the skeleton. The majority of the bones of the face and skull form via intramembranous ossification, in which undifferentiated mesenchymal cells convert directly into bone-forming osteoblasts. In contrast, the skeletal components of the axis, pelvis, and limbs form by endochondral ossification. Prior to condensation, mesenchymal cells secrete an ECM rich in hyaluronan and collagen I. It is known that the transcription factor Sox-9 (SRY-type high mobility group box-containing transcriptor factor) is essential for converting cells of condensations into chondrocytes, and it acts further at every stage of chondrocyte differentiation. Bone morgphogenetic proteins (BMPs), members of the transforming growth factor-β (TGF-β) family, also have an important role in the formation of mesenchymal condensations as shown by the finding that various inactivating mutations in the *Bmp5* gene lead to either several abnormal or absent condensations in short ear mice (Kingsley *et al.*, 1992). As the differentiation of chondrocytes begins as a result of the interactions of multiple signalling pathways, these developing chondrocytes begin to produce cartilage-specific proteins, such as collagens II, IX and XI, and aggrecan, while the expression of collagen I is turned off. The cartilaginous anlagen elongate and expand in width as a result of chondrocyte proliferation and the deposition of ECM. Chondrocytes in the central region undergo maturation to hypertrophic chondrocytes shortly after their formation. These cells start producing a differently composed ECM characterized by the expression of collagen X and angiogenic factors such as vascular endothelial growth factor (VEGF), which induce sprouting angiogenesis from the perichondrium surrounding the cartilaginous anlage. Osteoblasts, osteoclasts, and hematopoietic cells arrive along with the blood vessels. In these primary ossification centers, the hypertrophic cartilage is degraded, hypertrophic chondrocytes undergo apoptosis, and in the end of this process, cartilage is replaced by bone. Simultaneously, a collar of compact bone is formed by osteoblasts in the perichondrium encapsulating the primary ossification center, which is then located inside a bone tube and the diaphysis of bone is formed. (For more detailed review see DeLise *et al.* 2000, Olsen *et al.* 2000, Kronenberg 2003.)

The processes of joint specification or patterning (those which determine where a joint will form) and joint cavity formation (how a joint will form) both require precise regulation. The idea that joint cavity formation occurs within an apparently uninterrupted extracellular matrix was first presented as early as in the late 19th century (Bernays 1878). The synovial joints form between opposing discrete regions of mesenchymal expansion, whose location and length are predetermined by previous limb patterning events. The mechanisms regulating joint cavitation must lead to the formation of a non-adherent plane of cleavage, which involves both a local, precisely defined loss of tensile strength within the fragile interzone, where opposing skeletal elements meet, and the separation of

the cartilaginous surfaces by a matrix that facilitates their almost frictionless motion against one another. This interzone consists of three morphologically different layers: an intermediate looser cell layer consisting of mesenchymal cells, which becomes trapped between the ends of two outer chondrogenic layers consisting of densely packed mesenchymal cells at the interface of the interzone and the cartilaginous epiphyses. As the development progresses and the cartilaginous skeletal elements continue to expand, these interzonal regions become increasingly flattened.

It is now clear that the cavitation process does not appear to involve merely a liquification of the ground substance or cells, but rather a series of continuous, progressive changes in cell differentiation status leading to the distinct range of connective tissue of the joint (Lamb *et al.* 2003). There are two opinions of how the cavitation occurs. The first and still widely accepted theory suggests the formation of a partial cavity within the interzone as a result of local changes in the ECM and its enlargement by mechanical factors (Whillis 1940, Andersen & Bro-Rasmussen 1961, Murray & Drachman 1969, Doskocil 1985). The second one is based on selective cell degeneration and increased apoptosis within the interzone (Mitrovic 1977, 1978; Nalin *et al.* 1995, Abu-Hijleh *et al.* 1997). Some more recent studies (Ito & Kida 2000, Kavanagh *et al.* 2002) have concluded that apoptosis does not contribute to knee joint cavitation, but rather changes in the organization, shape, and contents of the interzone are essential. After the joint cavity has been formed, the cells in the outer chondrogenic layers of the interzone differentiate into chondrocytes that form the articular cartilage of the joint, while cells from the perichondrium differentiate and form the adjacent tendons and ligaments. (For a review, see Lamb *et al.* 2003.)

In recent studies the function of signalling pathways involved in the joint formation has been highlighted. Wnt-14, a secreted growth factor of the Wnt-gene family, is known to be highly expressed in joint-forming regions in the interzone and the neighbouring nonchondrogenic cells (Hartmann & Tabin 2001). This Wnt-14 expression seems to promote the interzone phenotype in prechondrogenic mesenchymal cells, and thus it has been suggested that Wnt-14 expression dictates the position of future joints. Another signalling pathway involved is the CDMP1/Gdf5 pathway. Normally Gdf5 (growth/differentiation factor 5) is expressed in developing joints throughout the cavitation process, and mutations in the genes coding for cartilage-derived morphogenetic protein 1 (*CDMP1*) and its mouse homolog, *Gdf5*, have been shown to cause defects in joint formation resulting in the shortening or missing of phalanges (for reviews, see Shum & Nuckolls 2002 and Lamb *et al.* 2003.) The molecular mechanisms of joint formation and articular chondrocyte differentiation are not currently fully understood, but the results of ongoing research will hopefully better describe the normal developmental processes in the future. (For reviews, see Shum & Nuckolls 2002 and Lamb *et al.* 2003.)

As the development of the skeleton continues after the formation of joints and the elongation of the cartilaginous anlagen, secondary ossification centers are formed within the chondroepiphysis at the ends of long bones distal to growth plates (Figure 1). This process is less well known when compared to the formation of primary ossification centers, but it is thought that the appearance of cartilage canals might participate in the initiation of secondary ossification centers. These canals contain blood vessels and penetrate from the perichondrium through the noncalcified cartilage matrix into the

avascular epihyseal cartilage, but their exact role is still unclear. Roach *et al.* (1998) suggested, based on their studies and the previous literature, that prior to the initiation of the ossification center, several cartilage canals converge around the future ossification center, and hypertrophy of the chondrocytes at this site is induced by factors diffusing from the canals. This is then followed by the same sequential steps, leading to the mineralization of cartilage as in the growth plate, which is described in more detail in the next chapter. As the secondary ossification centers are formed at the ends of the epiphyseal cartilage, a cartilaginous growth plate is left between them. The process of chondrocyte proliferation and differentiation in this growth plate then controls the longitudinal growth of the skeleton. (Ballock & O'Keefe 2003, Olsen *et al.* 2000.)

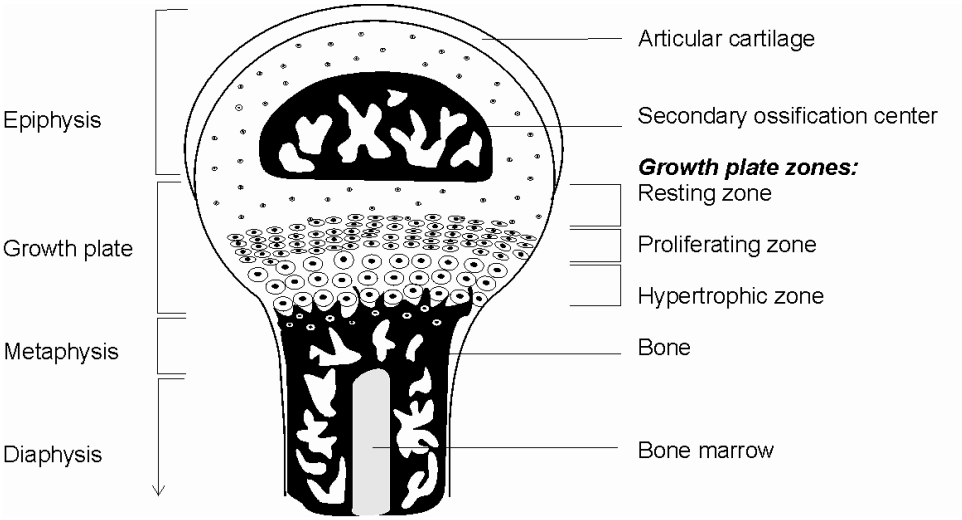

Fig. 1. Schematic presentation of a developing bone with different zones of grown plate shown.

2.3 Structure and function of the growth plate and epiphyseal cartilage

The growth plate can be divided into a series of anatomic zones (Figure 1) that represent unique morphological and biochemical stages during chondrocyte differentiation. The resting zone facing the epiphysis consists of chondrocytes which are in a relatively quiescent state, and it contains the highest proportion of ECM when compared to other zones of the growth plate. The next zone beneath the resting zone is the proliferating zone, where chondrocytes begin to divide, assuming a flattened appearance and becoming organized into columns. In the maturation zone, the rate of synthesis of ECM components is increased, allowing the separation of recently divided cells from each other. The hypertrophic zone consists of terminally differentiated chondrocytes, which

have increased cellular volume and increased alkaline phosphatase activity and collagen X expression. (Ballock & O'Keefe 2003.)

The general composition of this ECM is fairly similar to that of the ECM in the articular cartilage, consisting of collagens, proteoglycans, other noncollagenous proteins and water. The composition varies somewhat between different zones. The major collagen in the growth plate is collagen II (von der Mark & von der Mark 1977, Mizoguchi *et al.* 1990), and collagens IX and XI are also highly expressed (Mendler *et al.* 1989, Ballock & O'Keefe 2003). In addition, collagens VI, XII, and XIII have been found in the growth plate cartilage (Keene *et al.* 1988, Sandberg *et al.* 1989, Gregory *et al.* 2001). The principal proteoglycan in the growth plate is aggrecan (Mundlos *et al.* 1991), and other smaller proteoglycans such as decorin and biglycan are present (Heinegård & Oldberg 1989). COMP is found in human (Hecht *et al.* 2004), rat (Hedbom *et al.* 1992, Shen *et al.* 1995), bovine (Hedbom *et al.* 1992) and porcine (Ekman *et al.* 1997) epiphyseal and growth plate cartilage, and its strongest expression seems to be in the territorial matrix of the proliferative zone. Matrilin-3 is also expressed in all regions of the growth plate and epiphyseal cartilage both in humans (Kleemann-Fischer *et al.* 2001) and in mice (Klatt *et al.* 2000, Segat *et al.* 2000, Klatt *et al.* 2002, Ko *et al.* 2004), with the strongest expression being found in the territorial and interterritorial regions. The characteristic protein expressed by hypetrophic chondrocytes is collagen X (Schmid & Linsenmayer 1985).

Mineralization of cartilage ECM occurs in a somewhat directional pattern. Matrix vesicles, which are formed by the budding of the chondrocyte plasma membrane and deposited into the surrounding ECM of the hypertrophic zone, serve as a nidus for mineralization. Capillary loops from the metaphysis invade the mineralized cartilage, entering the lacuna of hypertrophic chondrocytes and bringing the osteoblasts, chondroclasts, and stromal cells needed for the completion of endochondral ossification. The growth plate is dependent on the diffusion of nutrients and oxygen from these vascular structures since the growth plate itself is avascular. The process of chondrocyte proliferation and differentiation in the growth plate controls the longitudinal growth of the skeleton. The rate of longitudinal bone growth diminishes as the developing skeleton approaches maturity, and the proliferation of growth plate chondrocytes decreases. Estrogen-mediated physeal closure occurs after puberty in humans and some other mammals; the growth plate is completely resorbed and the fusion of the epiphysis to the metaphysis occurs. (For reviews, see Ballock & O'Keefe 2003 and Olsen *et al.* 2000.) The molecular mechanisms of this closure are still being studied, but it has been suggested that estrogen might promote the programmed replicative senescence of growth plate chondrocytes (Weise *et al.* 2001).

Both *in vivo* and *in vitr*o studies using mouse models have shown that Indian hedgehog protein (Ihh), secreted by prehypertrophic and early hypertrophic chondrocytes in the developing bone, stimulates chondrocyte proliferation directly. Through the stimulation of parathyroid hormone-related protein (PTHrP) synthesis, Ihh determines the distance from the end of the bone at which chondrocytes stop proliferating and undergo hypertrophic differentiation. At this site, Ihh also has an effect on perichondrial cells, converting them into osteoblasts that form the bone collar. Many of the twenty-two fibroblast growth factor (FGF) genes and four FGF receptor genes are expressed during endochondral bone formation. To summarize the FGF studies, FGF signalling seems to

decrease chondrocyte proliferation both directly and by suppressing Ihh expression. BMP signalling antagonizes the effects of FGF signalling at several levels and promotes chondrocyte proliferation by increasing Ihh expression in prehypertrophic chondrocytes. In summary, chondrocyte proliferation during chondrogenesis and endochondral ossification is regulated by FGFs, BMPs, PTHrP, Ihh, cell-cell and cell-matrix adhesion and interactions, ECM components, and biomechanical signals. (For reviews, see Baitner *et al.* 2000, DeLise *et al.* 2000, Olsen *et al.* 2000, Shum & Nuckolls 2002, Horton 2003, and Kronenberg 2003). Various mutations have been characterized in the genes involved in these processes both in humans (reviewed by Superti-Furga *et al.*, 2001, Shum & Nuckolls 2002, Kornak & Mundlos 2003, Zelzer & Olsen 2003) and in mice (reviewed by McLean *et al.* 2001), which lead to disturbances in cartilage formation and endochondral ossification and result in various chondrodysplasia phenotypes.

2.4 Structure and function of articular cartilage

Articular cartilage is a hypocellular, aneural, alymphatic, and avascular tissue covering the ends of the bones in diarthrodial joints. The specific structural organization of the extracellular matrix produced by sparsely scattered chondrocytes is responsible for providing resistance to compressive forces, distributing load and, together with the synovial fluid, enabling frictionless movement of joints. The cell volume averages only approximately 1-2 % of the total cartilage volume in human adults and with aging, the number of chondrocytes and the production of the ECM decreases progressively. (Huber *et al.* 2000, Poole *et al.* 2001, Aigner & Stöve 2003.)

The organization of the ECM, chondrocytes and the proportion of different ECM components varies between the different zones of articular cartilage. There are four different horizontal layers: the superficial, transitional (intermediate), deep, and calcified zones (Figure 2). The superficial zone at the joint surface consists of flattened chondrocytes aligned parallel to the surface and surrounded by densely packed layers of thin collagen fibrils running parallel to each other and the surface. This zone, due to its collagen composition, provides the highest tensile properties found in articular cartilage. In the deeper zones, cell density decreases, while the diameter of collagen fibrils increases and their orientation becomes more random. Aggrecan content reaches its the maximum concentration in the deep zone. The chondrocytes in the calcified zone usually have a hypertrophic phenotype expressing collagen X, but this calcified matrix is not fully resorbed and provides structural integration with the subchondral bone. (For reviews, see Huber *et al.* 2000, Poole *et al.* 2001, and Aigner & Stöve 2003.)

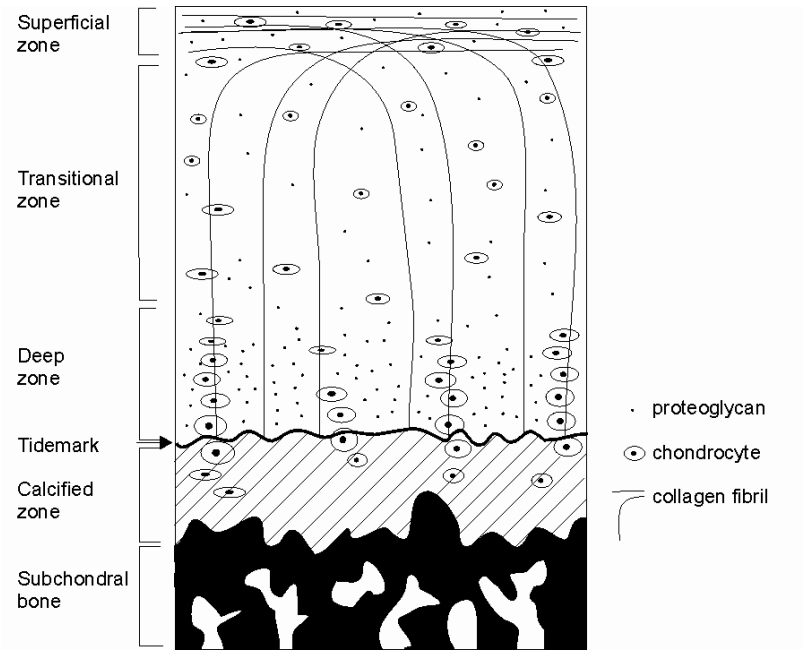

Fig. 2. Schematic presentation of the structure and zones of articular cartilage.

The ECM of articular cartilage is composed of tissue fluid, which is composed of water, dissolved ions, gases, small proteins, and metabolites, and macromolecules, which include collagens, proteoglycans, and noncollagenous proteins. Approximately 80 % of the weight of articular cartilage comes from water bound to proteoglycans, whereas collagens account for about 2/3 of the dry weight of cartilage. Collagen II is the major cartilage collagen forming heterotypic collagen fibrils with the minor cartilage collagens IX and XI (Huber *et al.* 2000, Poole *et al.* 2001, Aigner & Stöve 2003, Eyre 2004).

In addition, collagen X is expressed by chondrocytes in the calcified zone of normal articular cartilage. Collagen VI, ubiquitously found in most tissues, forms a branched filamentous network in the pericellular region of chondrocytes that can bind other ECM components of the pericellular matrix, such as decorin, fibromodulin, hyaluronan and fibronectin (Huber *et al.* 2000, Poole *et al.* 2001, Eyre 2002, Aigner & Stöve 2003). In addition, collagens III (Wotton & Duance 1994, Young *et al.* 2000), XII (Gregory *et al.* 2001), and XVI (Lai & Chu 1996) have been detected in articular cartilage, but they are not considered to be cartilage-specific as their main expression is in noncartilaginous tissues.

The major proteoglycan of cartilage is aggrecan, which consists of a protein core as well as keratan sulphate and chondroitin sulphate glycosaminoglycan chains. The hydration of these side chains provides the compressive stiffness of cartilage, and the subsequent swelling of the molecules is restricted by the fibrillar collagen network. The aggrecan monomers bind to hyaluronan and link protein to form large aggregates (Knudson & Knudson 2001). In addition, numerous non-collagenous proteins are expressed in articular cartilage, such as small proteoglycans containing leucine-rich

repeats (e.g decorin, biglycan, fibromodulin, and lumican), which have been shown to interact with the fibrillar collagens, perlecan, COMP, fibrilin-1, lubricin, chondroadherin, and proline-arginine-rich end leucine rich protein (PRELP) (see Roughley 2002, Poole *et al.* 2001, and Svensson *et al*. 2001.) Mutations in the genes encoding these proteins usually lead to disturbances in the developing cartilage or cartilage integrity as shown by mouse models and human chondrodysplasias (for reviews, see Aszódi *et al.*. 2000, Bateman 2001, Superti-Furga *et al.*, 2001, McLean & Olsen 2001, Helminen *et al.*. 2002, Shum & Nuckolls 2002, Kornak & Mundlos 2003, and Zelzer & Olsen 2003).

2.4.1 Collagenous components of cartilage

Collagens are a family of ECM proteins thal all consist of three polypeptide chains, called α chains, that are wrapped around each other into a triple helix. In some collagens all three α chains are identical, whereas in others all three α chains can be different. Each chain contains at least one domain composed of repeating –Gly-X-Y sequences, as well as glycine as every third amino acid. The presence of glycine, the smallest of amino acids, as every third amino acid is essential to the correct formation of the triple helix because a larger amino acid would not fit into the restricted space in the center of the triple helix. At least 27 vertebrate proteins with altogether 42 distinct α chains are now defined as collagens. Most collagens form supramolecular assemblies such as fibrils and networks, and traditionally collagens have been divided into fibrillar and nonfibrillar collagens. The group of non-fibrillar collagens has been further divided into several subgroups based on other structural and functional characteristics. The tissue distribution of collagens varies; while some are found only in specific tissues, such as collagen II in cartilage and cartilage-like tissues, others, eg. collagen VI, are ubiquitously expressed. (For reviews, see Myllyharju & Kivirikko 2001, Gelse *et al.* 2003 and Myllyharju & Kivirikko 2004.)

2.4.1.1 Collagen II

Collagen II is the most abundant and characteristic collagen of hyaline cartilage, but is also found in the vitreous humour of the eye, in the nucleus pulposus and in the annulus fibrosus of the intervertebral disks and inner ear. The *COL2A1* gene, located on chromosome 12q13.11-q13.12 (Takahashi *et al.* 1990), consists of 54 exons and codes for the three identical α chains of homotrimeric collagen II (Ala-Kokko & Prockop 1990, Ala-Kokko *et al.* 1995). The triple-helical domain consists of 1014 amino acid residues and is flanked by very short amino (N) and carboxy (C)-telopeptide domains at each end. The sequences coding for the N-terminal propeptide contain an alternatively spliced exon, and it has been shown that collagen IIA including the cysteine-rich domain is expressed in prechondrogenic and nonchondrogenic tissues during embryonic development. This domain acts by binding and regulating bone morphogenetic proteins, such as BMP-2 and TGF-β (Zhu *et al.* 1998). The collagen IIB form lacking this exon is the main form expressed in cartilage (Sandell *et al.* 1991). Collagen II forms the

backbone of the cartilage heteropolymeric fibrils, where collagen IX molecules are located covalently bound to the surface of the fibril and collagen XI is mostly located inside the fibrils (Figure 3). The features that make collagen II uniquely suitable for cartilage are unknown, but it has been suggested to be due to its high hydroxylysine content, which facilitates glycosylation, as well as its ability to interact with various other ECM components to form the fibrillar network. (Cremer *et al.* 1998, Eyre 2002.)

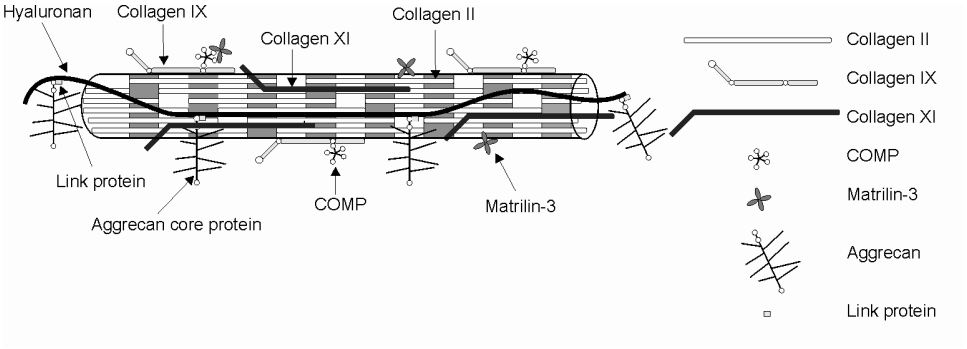

Fig. 3. Schematic presentation of cartilage ECM showing heterotypic collagen fibril, consisting of collagens II, IX, and XI, and its association with some of non-collagenous components of cartilage, such as aggrecan, COMP (cartilage oligomeric matrix protein, hyaluronan, link protein, and matrilin-3.

A variety of osteochondrodysplasias are caused by mutations in the *COL2A1* gene. As collagen II is expressed in the vitreous of the eye, the inner ear and the intervertebral disks, in addition to cartilage, patients with these disorders typically have myopia, sensorineural hearing defects, and spinal changes as well as a short stature and joint problems. The severity of these chondrodysplasias ranges from developmental or perinatal lethality (achondrogenesis type II, hypochondrogenesis), to moderately severe dwarphism (spondyloepiphyseal dysplasia (SED), Kniest dysplasia), to Stickler syndrome and precocious osteoarthropathy at the mild end of the spectrum. The most severe forms are caused by glycine substitution mutations leading to a total absence of collagen II from the cartilage matrix. The mildest phenotype, Stickler syndrome, is caused by premature stop codons leading to functional haploinsufficiency of collagen II (for reviews, see Kuivaniemi *et al.* 1997, Baitner *et al.* 2000, Bateman 2001, Myllyharju & Kivirikko 2001, Reginato & Olsen 2002, and Kornak & Mundlos 2003). Altogether 11 families with an R519C mutation in *COL2A1* leading to premature OA with only mild chondrodysplasia have been reported (Ala-Kokko *et al.* 1990, Pun *et al.* 1994, Williams *et al.* 1995, Holderbaum *et al.* 1996, Bleasel *et al.* 1998, Mier *et al.*. 2001, Moskowitz *et al.* 2004). In addition, four families with an R75C mutation leading to precocious OA and mild SED have been reported (Williams *et al.* 1993, Bleasel *et al.* 1995, Bleasel *et al.* 1996, Löppönen *et al.* 2004).

Several mouse lines with either a spontaneous deletion mutation in *Col2a1* (Pace *et al.* 1997) or transgenic and knockout models have been studied and reported in recent years (Table 1).

Table 1. Mouse models of Col2a1 mutations

Defect	Reference
Spontaneous mutations:	
dmm, 3 bp deletion in *Col2a1*	Pace *et al.* 1997
sedc, Col*2a1* R1417C (corresponds to *COL2A1* R789C)	Donahue *et al.* 2003
Transgenic mouse models:	
Targeted inactivation of *Col2a1*	Li *et al.* 1995
Deletion of 150 bp of exon 7 and intron 7 (Del1)	Metsäranta *et al.* 1992, Säämänen *et al.* 2000
Deletion of exon 48	Barbieri *et al.* 2003
Expression of human *COL2A1* with large internal deletion	Vandenberg *et al.* 1991, Helminen *et al.* 1993
Transgenic mouse models of point mutations:	
Col2a1 G85C mutation	Garofalo *et al.* 1991
Expression of human *COL2A1* with R519C mutation	Arita *et al.* 2002, Sahlman *et al.* 2004
Expression of human *COL2A1* with R789C mutation	Gaiser *et al.* 2002
Col2a1 G904C mutation	So *et al.* 2001

In mouse models of *Col2a1* mutations, homozygous transgenic mice carrying a defect listed in Table 1 usually show a severe chondrodysplasia phenotype and die shortly before or after birth. However, the naturally occurring R1417C mutation in *Col2a1* (corresponding to the human *COL2A1* R789C mutation) results in recessive congenital SED in mice (sedc) (Donahue *et al*. 2003). It has also been shown for some of these mutations that heterozygous mice develop early-onset OA with a mild chondrodysplasia (for a review, see Helminen *et al.* 2002).

2.4.1.2 Collagen IX

Collagen IX belongs to the group of so-called Fibril-Associated Collagens with Interrupted Triple helices (FACIT collagens), and its tissue expression pattern is similar to collagen II. It is a heterotrimeric molecule consisting of three different α chains, that form three triple-helical domains (COL1-3) flanked by four globular noncollagenous domains (NC1-4). The molecule is covalently crosslinked to the surface of collagen II fibrils in an antiparallel manner and in addition, collagen IX molecules are cross-linked to each other (see Eyre *et al.* 2002, 2004 for reviews). It has been shown to also interact with COMP (Holden *et al.* 2001, Pihlajamaa *et al.* 2004). In addition, it can be classified as a proteoglycan since there is an attachment site for a chondroitin sulphate side chain in the NC3 domain of the α2(IX) chain (Huber *et al.* 1986, McCormick *et al.* 1987). The length of the NC3 domain differs in all three α chains, thus forming a kink in the molecule (McCormick *et al.* 1987). When the collagen IX molecule is attached covalently to the surface of collagen II fibrils, this kink allows the COL3 and NC4 domains to project away from the fibril surface (Bruckner *et al.* 1988, Vaughan *et al.* 1988). The NC4 domain of α1(IX) is larger than that of the α2(IX) and α3(IX) chains and is encoded by six additional exons of *COL9A1,* compared to the *COL9A2* and *COL9A3* genes. In addition, there are two forms of α1(IX): the long form transcribed using the

normal promoter and a short form transcribed using an alternative promoter in intron 6. The short form of α1(IX) is primarily expressed in the eye (See Brewton & Mayne 1994).

The collagen IX α chains, α1(IX), α2(IX) and α3(IX), are encoded by their respective genes as shown in Table 2.

Table 2. Genes encoding the collagen IX α chains

α chain	Gene	Location	Number of exons	Size of gene (kb)	References
α1(IX)	*COL9A1*	6q12-13	38	90	Kimura *et al.* 1989, Warman *et al.* 1993, Pihlajamaa *et al*. 1998a
α2(IX)	*COL9A2*	1p32.3-p33	32	15	Perälä *et al.* 1993, Warman *et al.* 1994, Pihlajamaa *et al.* 1998a
α3(IX)	*COL9A3*	20q13.3	32	23	Tiller *et al.* 1998, Paassilta *et al.* 1999a

The function of collagen IX is not completely known, but it has been suggested to act as a macromolecular bridge between collagen fibrils and between collagen fibrils and other ECM components. It also seems to have a role in endochondral ossification and in the maintenance of ECM integrity in cartilage and intervertebral discs as mutations in genes encoding different α chains of collagen IX lead to MED (See section 2.6.2.2) and intervertebral disc disease in humans (Annunen *et al.* 1999a, Paassilta *et al.* 2001, Solovieva *et al.* 2002). Mice lacking the α1(IX) chains (Fässler *et al.* 1994, Hagg *et al.* 1997, Aszódi *et al.* 2000) and transgenic mice heterozygous for a large in-frame deletion of *Col9a1* (Nakata *et al.* 1993, Kimura *et al.* 1996) had otherwise normal skeletal development but developed precocious OA. The mice homozygous for the large in-frame deletion of *Col9a1* showed, in addition to precocious OA, mild chondrodysplasia with spine involvement and ophthalmopathy (Nakata *et al.* 1993).

2.4.1.3 Collagen XI

Collagen XI is a member of the family of fibrillar collagens (Morris & Bächinger 1987). It is a heterotrimer composed of three distinct α chains, α1(XI), α2(XI) and α3(XI) (Eyre & Wu 1987). The α1(XI) and α2(XI) chains are encoded by *COL11A1* and *COL11A2* genes, respectively, while the α3(XI) chain is an overglycosylated form of the IIB splicing variant encoded by the *COL2A1* gene (Eyre & Wu 1987, Wu & Eyre 1995). The *COL11A1* gene is located on chromosome 1p21 (Henry *et al.* 1988), while the *COL11A2* gene is located on chromosome 6p21.3 (Hanson *et al.* 1989, Law *et al.* 1990). The gene and protein structures of α1(XI) and α2(XI) are fairly similar to each other and to the α chains of collagen V (Fichard *et al.* 1994). In cartilage, collagen XI forms fibrils mainly composed of α1(XI), α2(XI) and α3(XI), whereas hybrid fibrils with collagen V exist in articular cartilage and in noncartilaginous tissues (Kleman *et al.* 1992, Mayne *et al.* 1993).

The α1(XI) and α2(XI) chains both contain similar N-terminal regions consisting of an N-propeptide domain, a variable region where alternative splicing occurs, a minor triple-helical domain (COL2), and a short noncollagenous telopeptide domain (NC2) (Fichard *et al.* 1994, Morris *et al.* 2000, Gregory *et al.* 2000). The major triple-helical region is about 1000 amino acids long, and the C-propeptide region of both α chains is highly conserved. Unlike other fibrillar collagens, some of the collagen XI molecules retain an N-terminal noncollagenous domain in their fully processed form (Thom & Morris 1991, Rousseau *et al*. 1996), whereas the cleavage of some collagen XI molecules occurs between Ala^{253} and Gln^{254}, seven residues before the end of this proline/arginine- rich N-propeptidedomain in chickens and rats (Rousseau *et al.* 1996, Medeck *et al.* 2003). It has been shown that Bmp-1 can cleave different isoforms of α1(XI) in rat (Medeck *et al.* 2003). This cleavage is likely to occur at the same site also in humans since this region of the cDNA is highly conserved (Rousseau *et al.* 1996). Thus, the variable region and COL2 domain are an integral part of the fibril and have been shown to be located on the surface of heterotypic fibrils (Keene *et al.* 1995, Gregory *et al.* 2000, Morris *et al.* 2000).

The *COL11A1* gene consists of 68 exons and spans over 150 kb (Annunen *et al.* 1999b). Alternative splicing of exons 6A, 6B, and 8 occurs, giving rise to formation of the six distinct isoforms with different spatial and temporal distributions during development and in mature tissues (Zhidkova *et al.* 1995, Oxford *et al.* 1995, Morris *et al.* 2000). The peptides encoded by exons 6A and 8 are acidic (pI 3.4), while exon 6B encodes a very basic peptide (pI 11.9) (Oxford *et al.* 1995, Zhidkova *et al.* 1995). The combination of exons 6A-7-8 appears to be the predominant and default splicing form in noncartilaginous tissues (Oxford *et al.* 1995, Yoshioka *et al.* 1995). The most abundant splice forms in mature cartilage are forms containing exons 6B-7 or containing only exon 7. (Chen *et al.* 2001). Expression of splice form p6b seems to be restricted in rat to the cartilage in the diaphysis adjacent to the perichondrium (Morris *et al.* 2000). It is likely that the different physiological properties of these isoforms could result in different interactions between different ECM components and the α1(XI) isoforms during development, since this variable region is located on the surface of heterotypic fibrils. This proposed function might explain the specific tissue distribution of the isoforms (Gregory *et al.* 2000, Morris *et al.* 2000, Chen *et al.* 2001). Despite the differential localization of the p8 and p6b isoforms in developing rat cartilage, no significant difference in fibril diameter was noted when fibrils from different regions were compared (Morris *et al.* 2000).

The *COL11A2* gene contains 66 exons and spans 28 kb (Vuoristo *et al.* 1995). There is alternative splicing of exons 6-8, and it has been shown that these α2(XI) mRNA isoforms are differentially expressed during mouse development. The major isoform in cartilage lacks exons 6-8, whereas the isoform containing exons 6-8 has been found in nonchondrogenic tissues such as the calvarium and periosteum (Sugimoto *et al.* 1998). Human fetal tissues also show developmentally stage-specific expression of different α2(XI) mRNA splice forms (Lui *et al.* 1996).

In addition to cartilage, all collagen XI α-chains are co-expressed with collagen II in cartilaginous tissues such as the nucleus pulposus of intervertebral discs and the inner ear, while the α2(XI) chain is replaced by the α2(V) chain in the vitreous of the eye (Cremer *et al.* 1998). The expression of the collagen XI α chains has also been detected in several human fetal tissues including the calvaria, kidney, skeletal muscle, brain, long bones,

tendon, and lung (Lui *et al.* 1995). The α1 chain is expressed also in several fetal tissues of the chicken and mouse, such as odontoblasts, trabecular bones, the atrioventricular valve of the heart, tongue, the intestine and the otic vesicle (Nah *et al.* 1992, Yoshioka *et al.* 1995).

The organization of collagens II, IX and XI into heterotypic fibrils occurs in cartilage (for reviews, see Cremer *et al* 1998 and Eyre 2002, 2004). Collagen XI is mainly located within the fibrils and covalently crosslinked to collagen II, while the persisting N-terminal noncollagenous domain of collagen XI is extended to the surface of these fibrils. Collagen XI molecules are also covalently crosslinked to each other (Cremer *et al.* 1998, Eyre 2004). Collagen XI has an essential role in the assembly, organization, and development of cartilage as shown by the *cho*-mouse model, where mouse homozygous for a naturally occurring single nucleotide deletion leading to a frame shift and premature termination codon resulted in functional knockout. Collagen α1(XI) chains were absent from the cartilage of *cho/cho* mice; the collagen fibrils in these mice were abnormally thick and the ECM and cartilage unorganized (Li *et al.* 1995, Li & Olsen 1997). Collagen XI seems to also have a role in regulating cartilage integrity, since the heterozygous *cho*-mice show precocious osteoarthritic changes in their joints (Xu *et al.* 2003, Rodriquez *et al.* 2004). These functions seem to be mediated by the coordination of fibril diameter by collagen XI molecules either by steric hindrance or by the binding of other ECM components (Gregory *et al.* 2000). Currently it is known that collagen XI can interact with proteoglycans (Vaughan-Thomas *et al.* 2001).

Mutations in *COL11A1* have been shown to cause two dominantly inherited chondrodysplasias in humans, Stickler and Marshall syndromes (Richards *et al.* 1996, Griffith *et al.* 1998, Annunen *et al.* 1999b, for reviews, see Baitner *et al.* 2000 and Kornak & Mundlos 2003) as well as non-syndromic Robin sequence (Melkoniemi *et al.* 2003).

The phenotypic spectrum caused by mutations in *COL11A2* ranges from dominant and recessive chondrodysplasias (Vikkula *et al.* 1995, van Steensel *et al*. 1997, Pihlajamaa *et al.* 1998b, Sirko-Osadsa *et al.* 1998, Melkoniemi *et al.* 2000, Vuoristo *et al.* 2004, Harel *et al*. 2005) to non-syndromic hearing loss (McGuirt *et al.* 1999). In addition, an allele of *COL11A2* has been shown to be associated with the ossification of the posterior longitudinal ligament of the spine (OPLL), which is the major cause for spinal stenosis in Japan (Maeda *et al.* 2001a, 2001b). The same association was reported also in Finnish spinal stenosis patients (Noponen-Hietala *et al.* 2003). A number of mutations have been charaterized in patients with otospondylomegaepiphyseal dysplasia (OSMED), a recessively inherited chondrodysplasia with severe sensorineural hearing loss and phenotypic overlap with Stickler and Marshall syndromes. However, OSMED patients do not have the ocular phenotype as the α2(XI) chain is not expressed in the eye (Vikkula *et al.* 1995, van Steensel *et al*. 1997, Melkoniemi *et al.* 2000, Harel *et al*. 2005). *COL11A2* mutations have also been found in the dominantly inherited form of non-ocular Stickler syndrome (sometimes referred to as heterozygous OSMED) (Vikkula *et al.* 1995, Pihlajamaa *et al.* 1998b, Sirko-Osadsa *et al.* 1998, Vuoristo *et al.* 2004). Mice homozygous for the disrupted *Col11a2* gene lack the full-length α2(XI) chain and show a chondrodysplasia phenotype with severe deafness comparable to OSMED in humans; heterozygous mice have no discernible abnormal phenotype by the age of one year (Li *et al.* 2001).

2.4.2 Noncollagenous components of cartilage

The ECM of cartilage contains a number of noncollagenous proteins, including aggrecan, small leucine rich proteoglycans, cartilage oligomeric matrix protein, and matrilin-3 (Huber *et al*. 2000, Poole *et al.* 2001, Eyre 2002, Aigner & Stöve 2003). A review of some of these proteins is presented in the following sections.

2.4.2.1 Cartilage oligomeric matrix protein

Cartilage oligomeric matrix protein (COMP) is a pentameric glycoprotein composed of five identical 110 kDa monomers (Hedbom *et al.* 1992, Oldberg *et al.* 1992, Mörgelin *et al.* 1992), and it is found predominantly in the ECM of cartilage (DiCesare *et al.* 1994a), tendons (DiCesare *et al.* 1994b, Smith *et al.* 1997), and ligaments (Muller *et al.* 1998). The human *COMP* gene is located on chromosome 19p12 (Newton *et al.* 1994) and consists of 19 exons encoding a protein of 757 amino acids (Briggs *et al.* 1995). COMP consists of a pentamerizing coiled-coil domain at the N-terminus followed by four type II (epidermal growth factor (EGF)-like) repeats, eight calcium-binding type III (calmodulin-like, T3) repeats, and a globular C-terminal domain (Oldberg *et al.* 1992, Briggs *et al.* 1995, Malashkevich *et al.* 1996). It is the fifth member of the thrombospondin protein family (Oldberg *et al.* 1992, Newton *et al.* 1994, Adams 2001), and its type III repeats, unique to thrombospondins, have been shown to bind calcium-ions (Ca^{2+}) cooperatively and with high affinity (Chen *et al.* 2000). It has been shown that COMP can interact with fibrillar and nonfibrillar collagens (collagens I, II, III and IX) with high affinity (Rosenberg *et al.* 1998, Holden *et al.* 2001, Thur *et al.* 2001, Pihlajamaa *et al.* 2004) as well as with fibronectin (DiCesare *et al.* 2002), at least *in vitro* via its C-terminal domain. In addition, COMP can interact with all matrilins, but the precise site for these interactions is not yet characterized (Briggs *et al.* 2002, Mann *et al.* 2004). The interactions with collagens are divalent cation-dependent, and the putative collagen binding site in COMP is located between residues 579-595 (Holden *et al.* 2001). An RGD cell-binding motif is present in the 3rd T3-repeat of human COMP, but its function is still unknown (Newton *et al.* 1994). Also there are three potential sites for N-linked glycosylation (Asn^{101}, Asn^{124}, Asn^{721}), of which Asn^{124} appears to be unsubstituted in humans (Zaia *et al.* 1997). The five-stranded N-terminal coiled-coil domain forms a continuous axial pore with binding capacities for hydrophobic compounds including prominent cell signalling molecules such as vitamins D and A, and all-trans retinol (Malashkevich *et al*. 1996, Guo *et al.* 1998, Özbek *et al.* 2002).

Mutations in *COMP* have been identified in two human chondrodysplasias, pseudoachondroplasia (PSACH) and MED, and these mutations are clustered within exons 8-19 in regions encoding either T3-repeats or the C-terminal domain (for reviews, see Briggs & Chapman 2002 and Chapman *et al.* 2003). Some of these mutations have been shown to affect the normal protein structure and calcium-binding of COMP, leading to accumulation of COMP, collagen IX, and matrilin-3 in the rough endoplasmic reticulum (rER) of chondrocytes. This contributes to the abnormal assembly and organization of the ECM (Hecht *et al.* 2005). The knockout mouse model of COMP

showed that the absence of COMP did not affect normal growth or development as homozygous and heterozygous mice did not differ from the wild-type mice (Svensson *et al.* 2002). This suggests that the lack of COMP is better than the presence of mutated COMP for skeletal growth and maintenance (Hecht *et al.* 2004). Even though the biological function of COMP is not yet fully known, it is likely to play an essential role in the assembly, organization, and maintenance of ECM in both developing and mature cartilage based on its interaction capacities, its role in PSACH and MED and its structural characteristics Furthermore, COMP may also serve a storage and delivery function for signalling molecules relevant to cartilage tissue. (Hecht *et al.* 2004, 2005.)

2.4.2.2 Matrilin-3

Matrilin-3, first isolated from chicken and mouse cartilage (Belluoccio & Trueb 1997, Wagener *et al.* 1997), is a member of the matrilin protein family, four of which (matrilin-1, -2, -3 and -4) are ECM proteins with a modular structure consisting of a vonWillebrand factor A-like (vWFA) domain and EGF-like domains, as well as a C-terminal α-helical coiled-coil oligomerization domain (Deák *et al.* 1999, Segat *et al.* 2001). In addition to matrilins, numerous other ECM components including collagens VI, VII, XII, and XIV, and the α-chains of seven integrins have been reported to contain vWFA domains (Deák *et al.* 1999). Human matrilin-3 is encoded by the *MATN*3 gene located on chromosome 2p24-p23, and the coding sequence of *MATN3* is organized into eight exons spanning approximately 21 kb, encoding a protein of 486 amino acids (Belluoccio *et al*. 1998). The protein consists of seven domains: a signal peptide, a single vWFA domain followed by four EGF-repeats, and a coiled-coil oligomerisation domain (Belluoccio *et al.* 1998). Matrilin-3 expression is restricted to cartilage, cartilaginous tissues, and bones in humans (Belluoccio *et al.* 1998, Kleemann-Fischer *et al.* 2001, Pullig 2002) and in mice (Segat *et al.* 2000, Klatt *et al.* 2000, Klatt *et al.* 2002). It is present in various types of cartilage such as developing cartilaginous anlage, growth plates, epiphyseal and articular cartilage as well as subchondral bone. It has been shown that matrilin-3 forms both homo-oligomers, preferentially homotetramers, and heterotetramers with matrilin-1 in human (Kleemann-Fischer *et al.* 2001), bovine (Wu & Eyre 1998, Klatt *et al.* 2000), mouse (Klatt *et al.* 2002) and chicken cartilage (Zhang & Cheng 2000), giving rise to a bouquet-like structure. These proteins are often co-localized in tissues, though clear differences in their spatial distribution have been observed (Kleemann-Fischer *et al.* 2001, Klatt *et al.* 2002).

It has been shown that matrilin-3 can interact with COMP (Mann *et al.* 2004, Briggs & Chapman 2002), and interactions between matrilin-1 and fibrillar collagens (Winterbottom *et al.* 1992) as well as between matrilin-1 and aggrecan (Hauser *et al.* 1996) have been reported. In a recent study, biglycan/matrilin-1 or decorin/matrilin-1 complexes were shown to act as linkers between collagen VI microfibrils and aggrecan or alternatively with collagen II; matrilin-3 was also found in these complexes (Wiberg *et al.* 2003). Thus, it is thought that matrilin-1 and -3 have an adapter function in the ECM connecting macromolecular networks. The importance of matrilin-3 in performing this task in cartilage is highlighted by the finding of mutations in a region encoding the

vWFA-domain in MED patients (Chapman 2001, Mostert *et al.* 2003, Jackson *et al.* 2004, Mabuchi *et al.* 2004). In addition, a polymorphism causing increased susceptibility to hand OA has been reported (Stefánsson *et al.* 2003). *Matn3* knockout mice, however, appear to be normal without any evidence of skeletal disorders (Ko *et al.* 2004). This suggests that *MATN3* mutations are likely to alter the folding or function of matrilin-3 and thus contribute to evident ECM disorganization via a dominant-negative effect.

2.4.2.3 Diastrophic dysplasia sulphate transporter

Diastrophic dysplasia sulphate transporter, DTDST, also known as SLC26A2 is an anion transporter acting as an Na^+-independent sulphate/chloride antiporter (Hästbacka *et al.* 1994, Satoh *et al.* 1998). It is a member of the newly delineated SLC26 anion transporter gene family (for a review, see Dawson & Markovich 2005), currently consisting of six known homologous human genes: liver sulphate anion transporter *SLC26A1* (also known as *SAT-1*) (Lohi *et al.* 2000), the major intestinal chloride/bicarbonate or chloride/hydroxide exchanger *SLC26A3* (alias *CLD* or *DRA*), which causes congenital chloride diarrhea when defective (Höglund *et al.* 1996), *SLC26A4* (alias *PDS*), a chloride/iodide or chloride/formate exchanger which causes Pendred syndrome when defective (Everett *et al.* 1999, Scott *et al.* 1999, Scott & Karniski 2000), *SLC26A5* (alias prestin) (Zheng *et al.* 2000), and *SLC26A6*, a pancreatic anion exchanger (Lohi *et al.* 2000).

The human *DTDST* gene was mapped to chromosome 5q32-q33.1 by positional cloning (Hästbacka *et al.* 1994) during the search for the gene responsible for diastrophic dysplasia, and the full transcript was expected to be 8.4 kb (Hästbacka *et al.* 1994, Haila *et al.* 2001). The coding sequence of *DTDST* is organized in two exons and encodes a protein of 739 amino acids (Hästbacka *et al.* 1994). The *DTDST* gene contains a large 3' UTR region of approximately 5.6 kb, and the 5'-UTR sequence was found to have an additional exon; a mutation affecting the splice donor site of this exon was found to be the founder mutation in Finnish diastrophic dysplasia patients (Hästbacka *et al.* 1999).

The DTDST protein is predicted to have 12 transmembrane domains as well as a C-terminal, cytoplasmic, moderately hydrophobic domain (Hästbacka *et al.* 1994). The function of the carboxy-terminal hydrophobic domain is not known, but is shows homology to bacterial proteins known as antisigma-factor antagonists, which are kinase antagonists and themselves kinase substrates (Aravind & Koonin 2000). Although the main expression of DTDST is detected in the developing hyaline cartilage, it is also expressed in the colon, placenta, bronchial glands, tracheal epithelium, pancreas, and eccrine sweat glands in humans. Its expression was almost absent from mature articular cartilage (Haila *et al.* 2001).

Mutations in the *DTDST* gene have been shown to cause four recessively inherited osteochondrodysplasias: lethal achondrogenesis type 1B (ACG1B), lethal atelosteogenesis type 2 (AO2), non-lethal diastrophic dysplasia (DTD), and nonlethal recessive multiple epiphyseal dysplasia (rMED). (For a review, see Rossi & Superti-Furga 2001). Depending on the location and type of the mutation, mutations either abolish or diminish the function of the sulphate transporter, leading to undersulfation of

the newly synthesized proteoglycans both *ex vivo* and in cell culture, which may explain the histological and physical changes of cartilage and thus the abnormal development and growth of the skeleton (Rossi *et al.* 1996, Rossi *et al.* 1998, Superti-Furga *et al.* 1994). Despite its widespread expression (Haila *et al.* 2001), the predominance of the phenotype in cartilage may be explained by several factors such as the high rate of proteoglycan synthesis and thus of sulphate requirement, the reduced vascular supply of thiols, and perhaps the low rate of thiol oxidation in cartilage (Rossi & Superti-Furga *et al.* 2001). Recently a knock-in mouse model was generated with a partial loss of function of the Dtdst sulphate transporter due to a human A386V mutation; additionally the presence of a *neomycine* cassette within intron 2 resulted in a reduced level of correctly spliced transcripts through the induction of abnormal splicing. The homozygous mice were characterized by growth retardation, skeletal dysplasia, and joint contractures closely resembling the human DTD phenotype (Forlino *et al.* 2005).

2.5 Osteoarthritis (OA)

OA is the most common joint disorder and is a major cause of morbidity and disability as well as a burden on health care resources. OA is no longer regarded as a simple consequence of ageing and cartilage degeneration, but as the result of active processes manifested by morphological, biochemical, molecular, and biomechanical changes of both cells and the ECM. These changes lead to the softening, fibrillation, ulceration, and loss of articular cartilage, sclerosis and the eburnation of subchondral bone, osteophytes, and subchondral cysts. As risk factors, pathophysiology, clinical features, and outcome vary from joint to joint, it has been suggested that OA may not be a single disorder but rather a group of overlapping distinct diseases. (Creamer & Hochberg 1997, Felson 2000, Buckwalter *et al*. 2004.)

2.5.1 Clinical features, diagnosis and classification of OA

OA most commonly affects the joints of the hands, knees, and hips. When OA becomes clinically evident, it is characterized by symptoms such as joint pain, tenderness, limitation of movement, crepitus, occasional effusion, and variable degrees of intra-articular inflammation without systemic effects. The clinical diagnosis of OA is usually confirmed by the finding of typical radiographic changes consisting of jointspace narrowing (JSN), the formation of marginal osteophytes, subchondral bone sclerosis, and subchondral cyst formation. As the jointspace narrowing becomes evident in x-rays only when cartilage loss is quite advanced, normal x-rays may not be sensitive enough to detect the early stages of OA. In addition, persons with evident radiographic changes due to OA may be totally asymptomatic. The symptoms best correlate with joint space narrowing, and sometimes the classification of OA into radiographic versus symptomatic OA is used. (For reviews, see Creamer & Hochberg 1997 and Buckwalter *et al*. 2004.)

Most OA patients suffer from the primary, idiopathic form of the disease where there is no evident predisposing factor. Secondary OA can develop as a consequence of

traumas, congenital or developmental defects (including chondrodysplasias and metabolic diseases), calcium deposition diseases (calcium pyrophosphate deposition disease, hydroxyapatite arthopathy), destructive artropathies (gout, rheumatoid arthritis), or miscellaneous other diseases including endocrine and neuropathic diseases. In addiation, OA can be classified as localized, affecting a certain joint, or as a generalized form of disease affecting three or more joint areas (Altman 1995, Creamer & Hochberg 1997, Buckwalter *et al*. 2004).

2.5.2 Prevalence of OA

The prevalence of OA in all joints is strongly correlated with age, with an almost exponential increase in prevalence after the age of 50 years. Regardless of how OA is defined, it is uncommon under the age of 40 years but prevalent in persons older than 60 years (Felson 2000, Buckwalter *et al*. 2004). It has been estimated that approximately 400 000 persons in Finland suffer from symptomatic knee or hip OA based on the findings of Mini-Finland and Health 2000 health examination surveys, where the diagnosis of hip or knee OA was based on a clinical examination and an interview about joint problems. Hip OA was diagnosed in 5 % of men and 4 % of women, and the corresponding figures for knee OA were 5 % for men and 7 % for women (Heliövaara 1996, Aromaa *et al.* 2002). A large recent population-based OA study evaluated the presence of radiological OA (ROA) in the hand, hip, knee, and thoracolumbar spine in 1355 Dutch subjects aged 55-65 years. Only 17 % of the studied persons were free from ROA in all joints; 61 % were found to have hand OA (at least one affected hand joint), 18 % had knee ROA, 11 % had hip ROA, and 69 % had disc degeneration in the spine (Zhai *et al.* 2004). Many population-based radiographic prevalence surveys for the hip OA have been performed. Most studies have been performed in Caucasian populations where the prevalence of ROA in the hip ranges from 1.6 % to 20 %, with an increase in prevalence with aging; hip ROA seems to be a little more common in men. (For a review, see Ingvarsson 2000). The most commonly affected joint is the hand, where OA usually affects the interphalangeal and first metacarpophalangeal joints. The prevalence of radiographic hand OA ranges from 64 % to 78 % in men over 65 years; the corresponding figures for women over 65 years are 71 % to 99 % (Mikkelsen & Duff 1970, Swanson & Swanson 1985, van Saase *et al.* 1989, Cauley *et al.* 1993, Haara *et al.* 2003).

2.5.3 Etiopathogenesis of OA

OA is currently considered a multifactorial disease, where genetic, local mechanical, and individual constitutional risk factors have an important role in the development of the disease.

The systemic risk factors for OA include a person's age, sex, ethnic characteristics, bone density, inherited susceptibility to OA, and other systemic factors, which probably operate by making cartilage more vulnerable to daily injuries and less capable of repair. Once systemic risk factors are in place, local biomechanical factors begin to play a role in

joint breakdown. These include obesity, joint injury, joint deformity, sports participation, and muscle weakness (For reviews, see Felson & Zhang 1998 and Felson 2000, 2004.)

OA has a higher prevalence and is more often generalized in women than in men. However, before the age of 50 years, men have a higher prevalence and incidence than women. The increase in the incidence and prevalence of OA with age is likely a consequence of several biological changes that occur with aging. Racial characteristics are also associated with OA susceptibility. The prevalence of hip OA seems to be low in Asians. In addition, hip OA is less prevalent in Blackfeet and Pima Native Americans. Several studies have shown that genetic factors are involved in OA susceptibility as reviewed in the next chapter. Evidence also suggests an inverse relationship between OA and osteoporosis. The preponderance of cross-sectional studies demonstrate that high bone mineral density is associated with an increased prevalence of hip, hand, and knee OA. In addition, some studies have reported an association between OA and the use of estrogen replacement therapy, vitamins C and D intake, and serum C-reactive protein levels. These findings, however, have been somewhat inconsistent. (For reviews, see Felson & Zhang 1998 and Felson 2000, 2004.)

Obesity increases the risk of developing knee OA, while being overweight also increases the risk for radiographic progression. The relationship between obesity and hip OA is not as strong as it is with knee OA. Although the relationships between acute joint trauma and the development of post-traumatic osteoarthritis remain poorly understood, it is clear that articular surface fractures, joint dislocations, and ligament and meniscal ruptures increase the risk of later OA, as do joint dysplasias. (For reviews, see Felson & Zhang 1998 and Felson 2000, 2004.)

OA involves the degeneration of articular cartilage together with changes in subchondral bone and limited intra-articular inflammation. Degeneration is first observed at the articular surface in the form of fibrillation, which involves splits more or less parallel to the articular surface. This superficial fibrillation is associated with the increased denaturation and loss of collagen II from collagen fibrils (Hollander *et al.* 1994, 1995). In addition, there is a local loss of aggrecan (Lark *et al.* 1997) and small proteoglycans, including decorin and biglycan (Poole *et al.* 1996). These changes result in an increase in water content and the loss of tensile strength in the cartilage matrix as the OA lesion progresses (Poole *et al.* 2002). The chondrocytes at the articular surface show increased expression of various matrix metalloproteinases (MMP) early in the degenerative process, which leads to the increased cleavage of collagen II and proteoglycans (Murphy *et al.* 2002). The collagenases implicated in collagen II degredation include collagenases 1, 2, and 3 (MMPs 1, 8, and 13, respectively) and membrane type 1 (MT1)-MMP (MMP14) (Mitchell *et al.* 1996, Billinghurst *et al.* 1997, Shlopov *et al.* 1997, Imai *et al.* 1997, Dahlberg *et al.* 2000). A major role in cartilage degradation has been suggested for MMP-13 due to its increased expression in OA cartilage and its ability to more effectively degrade type II collagen (Knauper *et al.* 1996, Mitchell *et al.* 1996, Wu *et al.* 2002). MMP-3, MMP-8, and MT1-MMP all can degrade aggrecan, but there is evidence that the primary event in “aggrecanase” cleavage is the preceding chondrocyte-mediated catabolism of aggrecan (Lark *et al.* 1997, Nagase & Kashiwagi 2003). Considerable data implicate a role for the synovium- and the chondrocyte-derived proinflammatory cytokines, interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), in the cartilage destruction associated with OA (Goldring & Goldring

2004). IL-1 and TNF-α have been shown to colocalize with MMPs in the superficial regions of OA cartilage (Tetlow *et al.* 2001). In addition, IL-1 and TNF- α can induce the expression of MMPs, aggrecanases, and other catabolic genes (Saklatvala 1986, Bunning & Russell 1989, Campbell *et al.* 1990, Lefebvre *et al.* 1990, Meyer *et al.* 1990) and inhibit the synthesis of proteoglycans and type II collagen by chondrocytes (Goldring *et al.* 1988, 1994; Chadjichristos *et al.* 2003), thus promoting chondrocyte dedifferentiation and preventing cartilage repair. However, the initial inducers of cartilage catabolism in OA have not been identified. Despite evidence of transient repair attempts by chondrocytes as shown by the increased expression of collagens IIA, III, VI, and X in the early OA lesion, the repair of matrix components is limited and, unless this process is interrupted, the damage to the cartilage matrix architecture cannot be regenerated by the adult articular chondrocyte (Poole *et al.* 2002). It has also been suggested that the loss of articular cartilage might reflect in part an insufficiency of anabolic growth factors such as insulin-like growth factor-1 (IGF-1) and transforming growth factor-β (TGF-β) (Trippel 2004). The TGF-β pathway is important in regulating cartilage turnover and maintaining healthy cartilage, as it has been shown that the articular cartilage degenerates in mice with a defective TGF-β type II receptor (Serra *et al.* 1997) or TGF-β driven SMAD signalling pathway (Yang *et al.* 2001). In addition, disruption of the normal pattern of expression of matrix components associated with certain genetic abnormalities can also predispose the cartilage to OA (Reginato & Olsen 2002). Despite active research work in the field of the molecular pathology of OA, the triggers of this cartilage destruction and the pathways used are still to be defined. (For reviews, see Poole 1999, Goldring 2000, Murphy *et al.* 2002, Poole *et al.* 2002, Goldring & Berenbaum 2004, Goldring & Goldring 2004, Martel-Pelletier 2004, and Trippel 2004.)

2.5.4 Inheritance of OA

Familial clustering of Heberden's nodes of the fingers was first formally studied by Stecher in the 1940s, when he discoverd the condition to be twice as frequent in the mothers and three times as frequent in the sisters of affected women when compared to the control population (Stecher 1941). Kellgren *et al.* (1963) reported same kind of familial clustering of generalized form of OA in the 1960's, reporting that the first degree relatives of patients with the generalized nodal OA were twice as likely to have radiologic evidence of the disease as the control population. These initial findings provided the rationale to investigate the inheritance and genetics of OA in more detail.

The development of hip OA seems to be strongly genetically determined. A British twin study estimated the heritability of hip OA to be approximately 60 % (MacGregor *et al.* 2000). Studies designed to evaluate the prevalence of hip OA among siblings of probands who have undergone total hip replacement (THR) have usually shown 2- to 3-fold increases in the risk of OA relative to controls (Lindberg 1986, Chitnavis *et al.* 1997, Ingvarsson *et al.* 2000), whereas the odds ratio for developing hip OA in siblings was 4.9 (95 % CI 3.9-6.4) in a large British study, where JSN <1.5 mm was used as a definition for probable hip OA (Lanyon *et al.* 2000).

Heritability estimates for knee OA have varied between 39-62 % (Spector *et al.* 1996, Neame *et al.* 2004). In a UK sibling study, the relative risk for total knee replacement (TKR) in siblings of probands compared to that found in the spouses of probands, who had undergone TKR, was 4.8 (95 % CI 0.64-36.4). However, the data was not adjusted for important risk factors, and the confidence intervals were wide because none of the controls had undergone TKR (Chitnavis *et al.* 1997). A more recent and thorough British study investigating the risk of tibio- and patellofemoral OA in siblings versus controls reported a relative risk of 2.9 (95 % CI 2.3-3.7) for tibiofemoral OA and 1.7 (95 % CI 1.4-2.2) for patellofemoral OA after adjusting for environmental factors (Neame *et al.* 2004). In addition, an MRI-based twin study showed that cartilage volume in different areas of knee joint were under genetic control, as the heritability for total knee cartilage volume was 73 % (95% CI 51-85%) (Hunter *et al.* 2003). These studies together confirm that genetic factors are likely to play an important role in the development of knee OA .

Hand OA and disc degeneration of the spine are also strongly influenced by genetic factors. Two studies have reported heritability values for hand OA at the same range, 56 % (95 % CI 0.34-0.76) in a Dutch sibling study (Bijkerk *et al.* 1999) and 59 % (95 % CI 0.49-0.70) in a British female twin study (Spector *et al.* 1996). Genetic factors seem to be important in disc degeneration in the spine, since the estimates of the heritability of disc degeneration have been approximately 70 % when based on normal radiographs (75 %, 95 % CI 0.30-1.00) or magnetic resonance imaging (74 %, 95 % CI 64-80 %) (Bijkerk *et al.*1999, Sambrook *et al.* 1999).

In addition, community-based segregation analyses of hand and polyarticular OA (Framingham and Baltimore longitudinal studies) have demonstrated a significant role for genetic factors in OA. The Framingham study suggested a Mendelian recessice inheritance pattern with a major recessive gene and a multifactorial component, representing either polygenic or environmental factors (Felson *et al.* 1998b, Hirsch *et al.* 1998). (For reviews, see MacGregor & Spector 1999, Loughlin 2001, Loughlin 2002a, Loughlin 2003, and Spector & MacGregor 2004.)

2.5.4.1 Association analysis of candidate genes in OA

As there is a wealth of evidence supporting the involvement of genetic factors in OA, the genetic basis of OA has been investigated by association studies of candidate genes or genome-wide linkage analysis of either affected sib-pairs or rare families where OA seems to be inherited in a Mendelian way. Numerous association studies have been performed since the late 1990's with a total number of over 50 candidate genes investigated, including genes encoding ECM components, genes affecting bone characteristics such as bone mineral density, and genes encoding catabolic and anabolic cytokines. The genes encoding ECM components have usually been genes in which mutations lead to more rare osteochondrodysplasia phenotypes with secondary early-onset OA of weight-bearing joints (for a review, see Reginato & Olsen 2002). Findings have rarely been consistent, possibly due to the use of different strategies in the studies. The most consistent finding is the involvement of polymorphisms in the vitamin D receptor gene (VDR) and in the interleukin-1 cluster in OA, with the relative risk being

approximately 2 as expressed in odds ratio (OR) (range 1.5-2.6) (Keen *et al.* 1997, Uitterlinden *et al.* 1997, Uitterlinden 2000, Loughlin *et al.* 2002b, Meulenbelt *et al.* 2004, Smith *et al*. 2004; for a review, see Spector & MacGregor 2004). Recently two association studies have reported the association of hip OA with the frizzled motif associated with bone development (*FRZB)* and the interleukin 4 receptor α (*IL4R*) genes, which were positional candidate genes based on the results of previously performed linkage analysis studies (Loughlin *et al.* 2004, Forster *et al.* 2004a). A Japanese group reported the association of an aspartic acid repeat polymorphism in the asporin gene (*ASPN*), a member of the small leucine-rich proteoglycan family, with knee and hip OA. In addition, they showed that asporin suppressed the TGF-β-mediated expression of aggrecan and collagen II in an *in vitro* model of chondrogenesis. This effect was allele-specific, with the OA-associated D14 allele resulting in greater inhibition than other alleles (Kizawa *et al.* 2005). Supplemental table 1 summarizes the results and characteristics of these association studies performed with hand, hip, or knee OA. (For reviews, see Brandi *et al.* 2001, Newman & Wallis 2002, Loughlin 2002a, Reginato & Olsen 2002, Loughlin 2003, and Spector & MacGregor 2004.)

2.5.4.2 Genome-wide linkage studies of OA

The genome-wide linkage studies of OA reported to date have been performed using either rare families where OA is inherited in an almost Mendelian manner, or using cohorts of affected sib-pairs. As the epidemiological studies (Lanyon *et al.* 2000, MacGregor *et al.* 2000, Spector & MacGregor 2004) have highlighted potential differences in the degree of OA heritability between different joint sites (hip, knee, and hand) and between sexes, most of the studies reported have stratified their data based on gender and the affected joint. Currently, there are results available from only one genome-wide linkage scan with hip and/or knee OA (Chapman *et al.* 1999, Loughlin *et al.* 1999, Loughlin *et al.* 2000, Chapman *et al.* 2002, Loughlin *et al.* 2002c, 2002d; Forster *et al.* 2004b, Loughlin *et al.* 2004), and the exclusion of l0 OA candidate genes (*COL2A1*, *VDR*, *COL9A2*, *COL11A1*, *COL11A2*, *HLA*, *COMP*, *CRTL1*, *α1AT*, *DTDST*) was performed prior to the genome-wide scan by analyzing the linkage of microsatellites in these OA families (Mustafa *et al.* 2000). Results from three genome-wide scans of hand OA have been reported (Leppävuori *et al.* 1999, Demissie *et al.* 2002, Stefánsson *et al.* 2003, Hunter *et al.* 2004).

To summarize the genome-wide linkage studies, multiple chromosomal regions have shown linkage with either hand or hip OA, and these findings are consistent with the heterogenous nature of OA. For hip OA, a linkage to overlapping regions of chromosome 16 has been reported by two different studies (Loughlin *et al.* 1999, Ingvarsson *et al.* 2001, Forster *et al.* 2004b), whereas linkages to chromosomes 2q24.3-q31.1, 4q13.1-4q13.2, 6p12.3-q13, 11q13.4-q14.3, and 11q21.1-q23.2 have not been replicated (Chapman *et al.* 1999, Loughlin *et al.* 1999, 2000; Chapman *et al.* 2002, Loughlin *et al.* 2002c,d, 2003; Forster *et al.* 2004b, Loughlin *et al.* 2004, Southam *et al.* 2004). However, evidence of the linkage of hand OA to chromosome 11q region overlaps with the linkage

of hip OA to 11q13.4-q14.3, suggesting that 11q is likely to harbour an OA susceptibility gene(s) (Demissie *et al.* 2002, Chapman *et al.* 2002).

In subsequent association analysis studies performed with candidate genes located in some of these regions, a UK group has reported the association of female hip OA with functional SNPs. Inheriting either the specific haplotype of the *FRZB* gene (OR 4.1), located in the 2q23-32 region, or the minor allele of two of the *IL4R* SNPs (OR 2.4), located on 16p12.1, seems to predispose females to hip OA (Loughlin *et al.* 2004, Forster *et al.* 2004a).

The most promising linkage of hand OA has been to the chromosome 2p24-p21 region detected by two large independent linkage studies (Demissie *et al*. 2002, Stefánsson *et al*. 2003). Furthermore, the association of hand OA with T303M variant in the *MATN3* gene located in this chromosomal region was reported by the Icelandic group (Stefánsson *et al.* 2003). Meanwhile, various other chromosomal regions warrant further studies (Demissie *et al.* 2002, Stefánsson *et al.* 2003, Hunter *et al.* 2004). The linkage of chromosomal region 2q to hand OA has been the most thoroughly studied, but despite the initial positive findings of Wright *et al.* (1996), the larger study by their group found no linkage of nodal hand OA (200 families) or knee OA (210 families) to chromosome 2q11.2-q36.3 (Gillaspy *et al.* 2002). Similar negative results of a linkage between 2q and hand OA have been obtained from an Australian study of 22 families with radiological hand OA (Stankovich *et al.* 2002). The only current study reporting positive linkage to this region is by Leppävuori *et al.* (1999). A study of 295 Russian nuclear families with radiological hand OA could not confirm the linkage of hand OA to chromosomal region 11q12-13 (Kalichman *et al.* 2003). A summary of OA linkage studies is shown in Table 3. (For reviews, see Loughlin 2002a, Loughlin 2003, and Spector & MacGregor 2004.)

Table 3. Linkage studies of hip and hand OA with suggestive linkage (LOD >1.5)

Chromosome	Position in cM (interval)	LOD-score	Joint or feature linked	Associated candidate gene	Country	Reference
Hip OA						
2q24.3-q31.1	175.5-184	MLS 1.6	female hip OA	*FRZB*	UK	Loughlin *et al.* 2002a, Loughlin *et al.* 2004
4q13.1-q13.2	79 (4 cM)	MLS 3.1	female hip OA	-	UK	Forster *et al.* 2004a
4q35	(11cM)	LOD 5.7	hip OA & dysplasia[a]	-	South Africa	Roby *et al.* 1999
6p12.3-q13	53-56	MLS 4.8	female hip OA	-	UK	Loughlin *et al.* 2002b, Southam *et al.* 2004
11q13.4-q14.3	71.6-83.5	MLS 2.4	female hip OA	-	UK	Chapman *et al.* 2002
11q21.1-q23.2	89.8-96.3	MLS 1.8	female hip OA	-	UK	Chapman *et al.* 2002
12q	56-68	MLS 3.2	early-onset hip/knee OA[b]	-	Finland	Palotie *et al.* 1989, Vikkula *et al.* 1993
16p	28-47	MLS 2.6	hip OA[a]	-	Iceland	Ingvarsson *et al.* 2001
16p12.3-p21.1	46 (12)	MLS 1.7	female hip OA	*IL4R*	UK	Forster *et al.* 2004a,
16q22.1-q23.1	89 (10)	MLS 1.9	female hip OA	-	UK	Forster *et al.* 2004a
19		MLS 1.6	hip OA[a]	-	Iceland	Ingvarsson *et al.* 2001
20		MLS 1.7	hip OA[a]	-	Iceland	Ingvarsson *et al.* 2001
Hand OA:						
1	30	LOD 1.5	MCP I	-	USA	Hunter *et al.* 2004
	102	LOD 3.0	JSN in hand	-	USA	Demissie *et al.* 2002
	218	LOD 2.1	DIP	-	USA	Hunter *et al.* 2004
2p	41.9-47.1	LOD 5.0	DIP/CMC 1	*MATN3*	Iceland	Stefánsson *et al.* 2003
	48	LOD 2.2	JSN in hand	-	USA	Demissie *et al.* 2002
2q12-q21	~116-155	LOD 2.3	DIP	-	Finland	Leppävuori *et al.* 1999
	181	LOD 1.6	Thumb IP	-	USA	Hunter *et al.* 2004
3	71	LOD 2.7	PIP	-	USA	Hunter *et al.* 2004
	96.8	LOD 1.8	DIP	-	Iceland	Stefánsson *et al.* 2003

Table 3. Continued.

Chromosome	Position in cM (interval)	LOD-score	Joint or feature linked	Associated candidate gene	Country	Reference
4q26-q27	~128	LOD2.3	DIP	-	Finland	Leppävuori *et al.* 1999
4q	155.9	LOD 3.3	DIP	-	Iceland	Stefánsson *et al.* 2003
7p21-p15	~13-39.5	LOD 1.4	DIP	-	Finland	Leppävuori *et al.* 1999
	50	LOD 2.3	JSN in hand	-	USA	Demissie *et al.* 2002
	155	LOD 3.1	DIP	-	USA	Hunter *et al.* 2004
	163	LOD 1.5	MCP I	-	USA	Hunter *et al.* 2004
8	105	LOD 2.1	DIP	-	USA	Hunter *et al.* 2004
9	76	LOD 2.3	JSN in hand	-	USA	Hunter *et al.* 2004
10	63	LOD 2.7	CMC I	-	USA	Hunter *et al.* 2004
	171	LOD 1.6	Thumb IP	-	USA	Hunter *et al.* 2004
11	77	LOD 1.6	JSN in hand	-	USA	Demissie *et al.* 2002
12	166	LOD 1.7	JSN in hand	-	USA	Demissie *et al.* 2002
	166	LOD 1.8	DIP	-	USA	Hunter *et al.* 2004
13	36	LOD 1.6	K/L sum in hand	-	USA	Demissie *et al.* 2002
	87	LOD 2.3	CMC I	-	USA	Hunter *et al.* 2004
14	118	LOD 1.6	CMC I	-	USA	Hunter *et al.* 2004
15	72	LOD 1.7	Thumb IP	-	USA	Hunter *et al.* 2004
	81	LOD 6.3	CMC I	-	USA	Hunter *et al.* 2004
17	22	LOD 2.2	CMC I	-	USA	Hunter *et al.* 2004
	100	LOD 2.3	DIP	-	USA	Hunter *et al.* 2004
19	52	LOD 1.8	JSN in hand	-	USA	Demissie *et al.* 2002
	68	LOD 1.8	K/L sum in hand	-	USA	Demissie *et al.* 2002

a Linkage study of one family; b Linkage study of two families. MLS = multipoint LOD score; LOD = logarithm of odds; CMC = carpometacarpal; DIP = distal interphalangeal, IP = interphalangeal, MCP = metacarpophalangeal; PIP = proximal interphalangeal; JSN = joint space narrowing; K/L = Kellgren/Lawrence

2.6 Multiple epiphyseal dysplasia (MED)

2.6.1 Clinical and radiological features of MED

Multiple epiphyseal dysplasia (MED) is a relatively mild, heterogeneous chondrodysplasia with a primarily autosomal dominant inheritance, although a recessive form (rMED) of the disorder also exists. It was initially recognized as a distinct chondrodysplasia by Sir Thomas Fairbank in 1947 (Fairbank 1947). A diagnosis of MED implies that radiographically there is a generalized abnormality of epiphyseal ossification without significant vertebral involvement. This can include a delay in ossification and abnormalities in epiphyseal size and contours. There is also an early-onset degenerative OA of the large weight-bearing joints (hips, knees), which suggests the presence of other mechanisms in addition to epiphyseal ossification abnormalities. In recent years, the molecular and genetic basis of MED has been under active research, and currently six different genes have been implicated in MED. (For reviews, see Unger & Hecht 2001, Briggs & Chapman 2002, Chapman *et al.* 2003, and Lachman *et al.* 2004). It is estimated that MED affects at least 1-4/10 000 individuals (Andersen *et al.* 1988).

MED patients usually have symptoms such as joint pain and stiffness, a waddling gait, easy fatique, and/or mild short stature in childhood. In addition, a MED diagnosis can sometimes be made based on the finding of early-onset OA of the knees and hips in adult patients. Patients with a severe form of the disease can be of short stature, while most patients have normal or mildly short stature. The radiological findings consist of initial delayed epiphyseal ossification, while later on ossification centers are usually small and sometimes fragmented with irregular contours. Usually the epiphyses of the hips and knees are the most severely affected, while ivory epiphyses of the hands and double-layered patella are other radiographic signs associated with the disorder. Spinal changes, if present, are mild and usually consist of Schmorl's nodes in the thoracolumbar vertebrae. In adulthood, the articular surfaces of the joints may be flattened and dysplastic with early-onset OA features that can be indistinguishable from the primary OA. Genu varum and genu valgum may also be present. (For reviews, see Unger & Hecht 2001, Briggs & Chapman 2002, Chapman *et al.* 2003, and Lachman *et al.* 2004).

Mutations in six different genes (*COMP, COL9A1*, *COL9A2*, *COL9A3, MATN3,* and *DTDST*) have been identified in MED patients, and some genotype-phenotype correlations have been suggested. MED patients with *COMP* mutations usually have the most severe changes in the hip joints, and in many cases the disease is progressive, leading to early-onset hip OA requiring joint replacement. Some patients with *COMP* mutations also have joint laxity. In contrast, the hip joints of patients with collagen IX mutations typically are relatively spared, while the most drastic radiographic findings are observed in the knees (Briggs & Chapman 2002, Unger *et al.* 2001). Myopathy in addition to epiphyseal dysplasia has been reported in two families with a *COL9A3* mutation (Bönnemann *et al.* 2000, Lohiniva *et al.* 2000). The hips and knees are the most commonly affected joints in patients with *MATN3* mutations, but the hip involvement is

intermediate relative to that caused by *COMP* or collagen IX mutations (Chapman *et al.* 2001, Mäkitie *et al.* 2004). The presence of epiphyseal changes in combination with a double-layered patella and/or clubfoot is characteristic of rMED caused by *DTDST* mutations (Superti-Furga *et al.* 1999, Rossi & Superti-Furga 2001, Ballhausen *et al.* 2003, Mäkitie *et al.* 2003).

2.6.2 Molecular genetics of MED

As the clinical phenotype is variable, the disorder is also genetically heterogenous. Mutations in the genes *COMP*, *COL9A1*, *COL9A2*, *COL9A3,* and *MATN3* genes have been shown to cause dominantly inherited MED, while patients with the recessive form of MED are either homozygous or compound heterozygous for mutations in the *DTDST* gene (Table 4).

2.6.2.1 Mutations in COMP

Mutations in the *COMP* gene were the first to be identified in MED (Briggs *et al.* 1995), and it has been shown that mutations in *COMP* cause a dominantly inherited form of MED (EDM1, OMIM 600310). Since then, altogether 22 different mutations in 25 probands have been described (Table 4); three of the mutations (D302V, D385N, T585M) have been recurrent. The majority of these MED-causing *COMP* mutations cluster in exons 8-14, which encode the calcium-binding T3-repeats, while the other mutations are found in exons 16-18, which encode the C-terminal globular domain (Briggs *et al.* 1995, Susic *et al.* 1997, Ballo *et al.* 1997, Loughlin *et al.* 1998, Igekawa *et al.* 1998, Briggs *et al.* 1998, Deere *et al.* 1999, Delot *et al.* 1999, Czarny-Ratajzack *et al.* 2001, Briggs & Chapman 2002, Kawaji *et al.* 2002, Mabuchi *et al.* 2003, Song *et al.* 2003). A majority of MED mutations have either been point mutations predicted to result in amino acid substitutions or small in-frame deletions or insertions (3-6 bp); only one mutation leading to a premature termination codon due to an out-of-frame insertion of 1 bp has been reported (Mabuchi *et al.* 2003). This suggests that mutations in *COMP* cause preferentially qualitative, not quantitative, defect (Chapman *et al.* 2003). Mutations in *COMP* also cause pseudoachondroplasia, a more severe chondrodysplasia, but the phenotypes are partly overlapping (Unger & Hecht 2001, Briggs & Chapman 2002).

The exact mechanism by which COMP mutations cause MED is not fully known, but the incorporation of mutant COMP monomers into COMP pentamers is likely to cause a dominant-interference effect with only 3 % of COMP pentamers being likely to consist of five normal monomers (Chapman *et al.* 2003). The cellular pathology of allelic PSACH has been more thoroughly investigated, and some data for MED pathology can be derived from these studies since similar kinds of mutations have been reported in MED (Chapman *et al.* 2003). It is known that mutations in the calcium-binding T3-repeats of COMP have a profound effect on the structure of this region, decreasing its ability to bind calcium, but these abnormal COMP monomers can be incorporated into pentamers (Delot *et al.* 1998, Chen *et al.* 2000, Maddox *et al.* 2000, Thur *et al.* 2001, Kleerokoper *et al.*

2002). Another mechanism by which dominant negative interference may occur is the accumulation of pentameric COMP in the rough endoplasmic reticulum of chondrocytes. Collagen IX, aggrecan and matrilin-3 have also been present in these RER vesicles (Stanescu *et al.* 1993, Maddox *et al.* 1997, Chapman *et al.* 2003, Hecht *et al.* 2004). Furthermore, normal interactions between COMP and other ECM components can be disturbed as a result of these mutations, and the altered binding of collagens I, II, and IX with mutated COMP has been demonstrated in the case of some mutations (Holden *et al.* 2001, Thur *et al.* 2001).

2.6.2.2 Mutations in COL9A1, COL9A2 and COL9A3

Mutations in the genes coding for the collagen IX α chains have been shown to cause a dominantly inherited form of MED, and altogether nine such mutations have been identified in 10 families, with one of the mutations being recurrent (Table 4). All these mutations cluster in the splice-donor or acceptor site of exon 3 of *COL9A2* (EDM2, MIM 600204) (Muragaki *et al.* 1996, Holden *et al.* 1999, Spayde *et al.* 2000, Fiedler *et al.* 2002) or *COL9A3* (EDM3, MIM 600969) (Paassilta *et al.* 1999b, Bönnemann *et al.* 2000, Lohiniva *et al.* 2000, Nakashima *et al.* 2005) or in the splice-donor site of exon 8 of *COL9A1* (Czarny-Ratajczak *et al.* 2001). These mutations result in the skipping of exon 3 of *COL9A2* and *COL9A3,* or exon 8 and/or 10 of *COL9A1,* during RNA splicing. The resulting 36-bp deletion at the mRNA level gives rise to a 12-amino acid in-frame deletion in the α2(IX) or α3(IX) chains. The deletion of 75, 63, or 138 bp in *COL9A1* is predicted to cause an in-frame deletion of 25, 21 or 49 amino acids from the α1(IX) chain. These deletions are all located in an equivalent region of the COL3 domain of collagen IX, suggesting an important function for this domain.

It is known that the COL3 and NC4 domains of collagen IX are projected away from the collagen fibril surface into the cartilage ECM, where they possibly interact with other matrix molecules. A deletion of 12 or more amino acids from this domain may alter the conformation of the molecule with subsequent changes in its ability to interact with other ECM molecules (Chapman *et al.* 2003). In addition, some collagen IX mutations have been reported to lead to decreased mRNA stability and incomplete splicing of exon 3 (Holden *et al.* 1999, Spayde *et al.* 2002). The cell and matrix pathology of MED resulting from collagen IX mutations is not yet thoroughly studied and much work remains to be done to fully understand the disease process.

2.6.2.3 Mutations in MATN3

A genome-wide screen of a family with autosomal dominant MED not linked to the *COMP*, *COL9A2,* and *COL9A3* genes provided significant evidence of a linkage to chromosome 2. Subsequently, the first mutation in *MATN3* was identified in this family, implicating the fifth gene for the autosomal dominant form of MED (EDM5, MIM 602019) (Chapman *et al.* 2001). Now a few years later, *MATN3* mutations seem to be a quite common cause of this relatively mild and possibly self-limiting form of MED, with

nine different mutations being reported in altogether 17 probands (Table 4); two of the mutations (T120M, R121W) have been recurrent (Chapman *et al.* 2001, Mostert *et al.* 2003, Jackson *et al.* 2004, Mabuchi *et al.* 2004). All are missense mutations located in the exon 2 of *MATN3,* affecting the β-sheet region of the vWFA domain, with the exception of the F105S mutation located within an α-helix region (Chapman *et al.* 2001, Mostert *et al.* 2003, Jackson *et al.* 2004, Mabuchi *et al.* 2004). As matrilins have been found in collagen-dependent and -independent filamentous networks and preliminary data has demonstrated that matrilin-3 can bind with high affinity to COMP, collagen II, and the collagenous regions of collagen IX, it is possible that these mutations modify the normal interactions of ECM components by altering the structure and/or function of the vWFA domain of matrilin-3, thus causing structural abnormalities within the ECM of cartilage (Chapman *et al.* 2003).

2.6.2.4 Mutations in DTDST

Mutations in the *DTDST* gene had previously been shown to cause more severe recessive chondrodysplasias, achondrogenesis 1B, atelosteogenesis type 2, and diastrophic dysplasia (for a review, see Rossi & Superti-Furga 2001). Superti-Furga *et al.* (1999) reported the first patient with a recessive form of MED (rMED) with molecular verification; the patient was found to be homozygous for the most common rMED mutation, R279W. The current number of reported probands being R279W homozygotes is 18 (Superti-Furga *et al.* 1999, Huber *et al.* 2001, Czarny-Ratajzak *et al.* 2001, Ballhausen *et al.* 2003). In addition, four other mutations ('Finnish' IVS1+2T>C, N77H, G237V, C653S) have been reported to result in rMED when either homozygous (C653S) or compound heterozygous (see Table 4) in six probands. Thus, *DTDST* mutations seem to be a common cause of MED, and these patients usually present with either clubfoot and/or double-layered patella in addition to epiphyseal dysplasia (Superti-Furga *et al.* 1999, Rossi & Superti-Furga 2001, Ballhausen *et al.* 2003, Mäkitie *et al.* 2003).

Table 4. Reported MED mutations by gene

Gene	Exon/ intron	Nucleotide variation	Predicted Protein change	Location	N of cases[a] Sp	Fam	Reference
COMP	8	c.827C>G	P276R	T3-1		1	Czarny-Ratajzak *et al.* 2001
	9	c.905A>T	D30^{2}V	T3-2		2	Deere *et al.* 1999
	10	c.1024G>C	D342Y	T3-3	1		Briggs *et al.* 1995
	10	c.1081G>T	D361Y	T3-4		1	Loughlin *et al.* 1998
	10	c.1082A>T	D361V	T3-4		1	Igekawa *et al.* 1998
	10	c.1098-1191del CGGGGC	del RG 367-368	T3-4	1		Loughlin *et al.* 1998
	10	c.1111T>A	C371S	T3-4		1	Susic *et al.* 1997
	10	c.1112G>T	C371F	T3-4		1	Mabuchi *et al.* 2003
	11	c.1153G>A	D385N	T3-4		1	Song *et al.* 2003
					1		Mabuchi *et al.* 2003
	11	c.1247G>T	D408Y	T3-5		1	Loughlin *et al.* 1998
	12	c.1284A>C	D420A	T3-6		1	Czarny-Ratajzak *et al.* 2001
	13	c.1358G>A	N453S	T3-7		1	Briggs *et al.* 1998
	13	c.1371-1373del	E457del	T3-7	1		Mabuchi *et al.* 2003
	13	c.1405-1419 insGAC	InsD 469-473	T3-7		1	Delot *et al.* 1999
	13	c.1418G>A	D473G	T3-7	1		Briggs *et al* .2002
	13	c.1467G>C	N489K	T3-8	1		Briggs *et al.* 2002
	14	c.1501-1509del	G^{501}_V^{503} del	T3-8	1		Mabuchi *et al.* 2003
	14	c.1569C>G	N523K	T3-8		1	Ballo *et al.* 1997
	16	c.1754C>G	T585R	Ct		1	Briggs *et al.* 1998
	16	c.1754C>T	T585M	Ct	1		Czarny-Ratajzak *et al.* 2001
					1		Song *et al.* 2003
	18	c.2152C>T	R718W	Ct		1	Mabuchi *et al.* 2003
	18	c.2223-2224insC	N742fs X743	Ct		1	Mabuchi *et al.* 2003
COL9A1	IVS8	IVS8 ds +3 ins T	del of 25, 21 or 47 aa	COL3		1	Czarny-Ratajczak *et al.* 2001
COL9A2	IVS3	IVS3 ds+2 T>C	del of 12 aa	COL3		1	Muragaki *et al.* 1996
	IVS3	IVS3 ds+5 G>C	del of 12 aa	COL3		1	Holden *et al.* 1999
	IVS3	IVS3 ds-1 G>A	del of 12 aa	COL3		1	Holden *et al.* 1999

Table 4. Continued.

Gene	Exon/ intron	Nucleotide variation	Predicted Protein change	Location	N of cases[a] Sp	Fam	Reference
	IVS3	IVS3 ds+6 T>G	del of 12 aa	COL3		1	Spayde *et al*. 2000
	IVS3	IVS3 ds-1 G>C	del of 12 aa	COL3		1	Fiedler *et al*. 2002
COL9A3	IVS2	IVS2 as-1 G>A	del of 12 aa	COL3		1	Lohiniva *et al.* 2000
						1	Bönnemann *et al.* 2000
	IVS2	IVS2 as-2 A>T	del of 12 aa	COL3		1	Paassilta *et al.* 1999
	IVS3	IVS3 ds+5 G>A	del of 12 aa	COL3		1	Nakashima *et al*. 2005
MATN3	2	c.314T>C	F105S	vWFA (α1)		1	Mabuchi *et al.* 2004
	2	c.359C>T	T120M	vWFA (βB)		1	Jackson *et al.* 2003
						3	Mabuchi *et al.* 2004
	2	c.361C>T	R121W	vWFA (βB)		1	Chapman *et al.* 2001
						3	Jackson *et al.* 2003
						3	Mabuchi *et al.* 2004
	2	c.368C>A	A123K	vWFA (βB)	1		Mabuchi *et al.* 2004
	2	c.382G>C	A128P	vWFA		1	Mostert *et al.* 2003
	2	c.400G>A	E134K	vWFA (βC)		1	Jackson *et al.* 2003
	2	c.575T>A	I192N	vWFA (βD)		1	Jackson *et al.* 2003
	2	c.581T>A	V194D	vWFA (βD)		1	Chapman *et al.* 2001
	2	c.656C>A	A219D	vWFA (βE)		1	Jackson *et al.* 2003
DTDST	2	c.862C>T	R279W	homoz.		1	Superti-Furga *et al.* 1999
						2	Huber *et al*. 2001
						2	Czarny-Ratajczak *et al.* 2001
						15[b]	Ballhausen *et al.* 2003
	3	c.1984T>A	C653S	homoz.		2	Ballhausen *et al.* 2003, Mäkitie *et al.* 2003
	IVS1/e 2	IVS1+2T>C / c.862C>T	Finnish / R279W	compound heteroz.		1	Ballhausen *et al*. 2003
	IVS1/e 3	IVS1+2T>C / c.1984T>A	Finnish / C653W	compound heteroz.		1	Ballhausen *et al.* 2003
	e2/ e2	c.256A>C / c.862C>T	N77H / R279W	compound heteroz.		1	Ballhausen *et al.* 2003
	e2/e2	NA / c.862C>T	G237V / R279W	compound heteroz.		1	Ballhausen *et al.* 2003

[a] Sp = sporadic; Fam = familial; homoz. = homozygous: heteroz. = heterozygous

[b] Includes the original proband reported by Superti-Furga *et al.* 1999

3 Outlines of the present study

As there is a wealth of evidence for the involvement of genetic factors in OA, association studies of candidate genes, or genome-wide linkage analysis of either affected sib-pairs or rare families where OA seems to be inherited in a Mendelian way, has been performed to find the susceptibility genes. Some previous association and linkage studies and transgenic mouse models had suggested that sequence variations in *COL2A1* might play a role in the pathogenesis of OA. It had already been demonstrated that mutations in the minor cartilage collagen genes (*COL9A1, COL9A2, COL9A3, COL11A1,* and *COL11A2*) resulted in milder chondrodysplasias with early-onset OA as a part of the phenotype. In addition, mice lacking the α1(IX) chains and transgenic mice heterozygous for a large in-frame deletion of *Col9a1* developed degenerative joint disease similar to human OA. When this study was initiated, no studies concerning the role of sequence variations in minor cartilage collagens in OA had been reported.

The phenotype of MED cases, especially in adulthood, is sometimes indistinguishable from that of primary OA and it was thought that characterization of the genetic defects in MED patients could help in the search for OA susceptibility genes. Although mutations had already been reported in six different genes in MED patients, these were thought to account for less than 50 % of MED cases. It was not yet evident what proportion of cases was due to mutations in each gene implicated in MED, and genotype-phenotype correlations were still not thoroughly known. None of the reported studies had analyzed the patients for all of these MED genes, and no cohort-based reports of the prevalence of mutations in these genes had been reported.

Based on the indirect evidence of the potential contribution of defects in minor cartilage collagens in OA pathogenesis, it was hypothesized that mutations in the cartilage collagen genes, especially those coding for minor cartilage collagens IX and XI, might play a role in OA pathogenesis. In addition, the characterization of genetic defects in clinically well-studied MED cases and in a cohort of MED cases would improve the knowledge of genotype-phenotype correlations and help to estimate the prevalence of the MED mutation. It might potentially lead to the characterization of a subset phenotype of MED patients who do not have mutations in the currently known genes, enabling further studies which could potentially identify new MED genes. The object of this work was therefore to increase our knowledge of OA pathogenesis by studying the role of the

sequence variations in cartilage collagen genes in OA patients with early-onset primary hip and/or knee OA and to further evaluate the genetics and genotype-phenotype correlations in MED. The specific aims were:

1. to study whether sequence variations in cartilage collagen genes (*COL2A1, COL9A1, COL9A2, COL9A3, COL11A1,* and *COL11A2*) contribute to OA pathogenesis by performing a mutation and association analysis of these genes in patients with primary early-onset hip and/or knee OA,
2. to search for a disease-causing mutation in two MED families where the phenotype was suggestive for a collagen IX mutation, and
3. to search for MED-causing mutations in the genes implicated in MED (*COMP, COL9A1, COL9A2, COL9A3, MATN3,* and *DTDST*) in a cohort of Canadian MED patients and to estimate the prevalence of the MED mutations in this cohort. In addition, we sought to evaluate the genotype-phenotype correlations both in patients with an identified genetic defect and in patients in whom no mutations in the known genes were identified to possibly characterize new subsets of MED phenotypes.

4 Materials and methods

The materials and methods are described in detail in the original papers I-III.

4.1 Patients and controls (I-III)

Seventy-two unrelated Finnish patients with early-onset (< 50 years) hip and/or knee OA from the Departments of Orthopaedics at Jyväskylä Central Hospital and Kuopio University Hospital were included in the mutation and association analysis study of the cartilage collagen genes. They had all undergone at least one hip or knee arthroplasty or other open or arthroscopic surgery of the knee joint (Table 1 in article I) in which the diagnosis of OA was confirmed in addition to clinical and radiographical examinations. Altogether 103 unrelated controls were obtained; 50 controls were from Jyväskylä Central Hospital and 53 from Kuopio University Hospital. They were all over 45 years of age and had no history or symptoms of OA. They were not examined clinically or radiologically. In addition, a North American family with early-onset OA was referred to us for mutation analysis and was included in this study.

Two small MED-families with a phenotype suggesting a collagen IX mutation were referred to us for mutation analysis, and all subjects were examined clinically and radiographically. The Canadian MED cohort consisted of altogether 30 consecutive unrelated patients who were followed by Dr. W.G. Cole at the Orthopaedic Clinic, The Hospital for Sick Children, Toronto, during the years 1996 to 2002. Detailed clinical and mutation data have been reported previously for four of these patients (Susic *et al.* 1997, Ballhausen *et al.* 2003, Mäkitie *et al.* 2003). Of the remaining 26 patients, 25 consented to participate in the study. Their hospital records were reviewed for symptoms, clinical features and family history, and radiographs were assessed for skeletal characteristics of MED. The diagnosis of MED was based on typical clinical and radiographic features by expert clinicians in both MED studies.

Informed consent was obtained from all of the study subjects or their parents, and genomic DNA was isolated from peripheral blood lymphocytes using standard protocols and used for analyses.

4.2 Conformation sensitive gel electrophoresis (CSGE) analysis (I-III)

The conformation-sensitive gel electrophoresis (CSGE) method (Ganguly *et al.* 1993, Körkkö *et al.* 1998, Annunen *et al.* 1999b, Ganguly 2002) was used to screen the candidate genes for mutations. The primers were designed to amplify the target sequence, which consisted of the exonic sequence and its corresponding flanking intronic sequences, by polymerase chain reaction (PCR) to obtain PCR products of 182 to 487 bp to be used for mutation screening by CSGE. The amplifications were carried out in a volume of 20 µl that contained 20 to 60 ng of genomic DNA, 5 to 10 pmol of the PCR primers, 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs, and one unit of AmpliTaq Gold DNA polymerase (Applied Biosystems). The PCR products were analyzed on 1.5 % agarose gels prior to CSGE analysis to estimate the quality and concentration of each product. The thermocycling and the CSGE analysis conditions were the same as described earlier (Annunen *et al.* 1999b), with the exception that the CSGE gels were stained with SYBR Gold nucleic acid gel stain (Eugene, USA) instead of ethidium bromide.

4.2.1 Mutation screening of the COL2A1, COL9A1, COL9A2, COL9A3, COL11A1, and COL11A2 genes in OA patients (I)

Sequences corresponding to all 52 exons of the *COL2A1* gene (Ala-Kokko *et al.* 1995), all 38 exons of *COL9A1* (Pihlajamaa *et al.* 1998a), all 32 exons of *COL9A2* (Pihlajamaa *et al.* 1998a), 31 of the 32 exons (exons 2-32) of *COL9A3* (Paassilta *et al.* 1999a), all 68 exons of *COL11A1* (Annunen *et al.* 1999b) and all 66 exons of *COL11A2* (Vuoristo *et al.* 1995), as well as the corresponding intronic flanking sequences, were amplified by PCR to obtain products of 182 to 487 bp. Each sample was analyzed by CSGE to reveal possible sequence variations in OA patients. The control samples were analyzed for the presence of sequence variations identified in OA patients in these genes using CSGE and sequencing when needed. If a patient was found to have a possibly pathogenetic mutation, all available family members of such patients were also analyzed for the presence of this sequence variation either by CSGE and sequencing or restriction enzyme analysis, and their clinical status was evaluated to study the co-segregation of the variation with the phenotype.

4.2.2 Mutation screening of the COL9A1, COL9A2, COL9A3, COMP, MATN3 and DTDST genes in MED patients (II, III)

Since all reported MED mutations in the collagen IX genes are clustered in the splice sites of exon 3 of *COL9A2* or *COL9A3* or the splice donor site of exon 8 of *COL9A1* (Briggs & Chapman 2002), sequences corresponding to exons 8 to 10 of *COL9A1*, exons 2 to 4 of *COL9A2* and *COL9A3*, as well as the flanking intronic sequences, were amplified by PCR to obtain products of 252 to 396 bp for mutation screening by CSGE

followed by direct sequencing of these samples. Sequences corresponding to all 19 exons and exon boundaries of *COMP* were amplified by PCR to obtain products of 259 to 401 bp and were analyzed by CSGE. As all *COMP* mutations causing MED have been reported to be located between exons 8 and 19 (Briggs & Chapman 2002), these exons and the flanking intronic sequences were also analyzed by direct sequencing of all samples in addition to CSGE analysis. Since the *DTDST* exons are large in size, these exons (1 to 3) and their flanking sequences were amplified by PCR in four fragments, which were analyzed for nucleotide variations by sequencing. Mutation analysis of all the exons and flanking intronic sequences of *MATN3* had been performed previously on 20 of the 25 patients and the results of this analysis reported earlier (Jackson *et al.* 2004, Mäkitie *et al.* 2004). As five additional patients had been ascertained since the initial analysis, *MATN3* exon 2 was amplified from their samples as previously described (Jackson *et al.* 2004) and analyzed for sequence variations by CSGE and sequencing. If a patient was found to have a possibly pathogenic mutation, all available family members of such patients were also analyzed for the presence of this sequence variation either by CSGE and sequencing or restriction enzyme analysis, and their clinical status was evaluated to study the co-segregation of the variation with the phenotype.

4.3 Sequencing of PCR products (I-III)

Sequencing of the PCR products showing heteroduplexes in CSGE analysis, PCR products containing certain MED hot-spot exons, or PCR products containing known SNPs to be genotyped for OA association analysis was carried out with an ABI PRISM™ 377 or 3100 Sequencer (Applied Biosystems) using either the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia) or the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). The PCR products had been treated prior to sequencing with exonuclease I and shrimp alkaline phosphatase to remove residual PCR primers and nucleotides (Hanke & Wink 1994, Werle *et al.* 1994).

4.4 RT-PCR (I, III)

RT-PCR analysis of two OA patients was performed to study the effect of the observed sequence variations (*COL11A2* IVS42-2del A, *COL2A1* c.2659G>A affecting the last nucleotide of exon 38) on splicing. RNA analysis of *COMP* in MED patients 7 and 9 was performed to search for possible mutations resulting in larger gene rearrangements that were not detected by CSGE and to confirm the negative findings of the mutation screening at the DNA level performed that was performed with CSGE and sequencing.

Total RNA was extracted from EBV-transformed lymphoblasts of a North American OA patient (subject II:2; Figure 1 in article I), from a Finnish OA patient (proband J35), and from MED patients 7 and 9 using either the TRIzol LS Reagent Kit (Gibco BRL) or the RNeasy kit (Qiagen) and used as a template for RT-PCR. The first strand was synthesized using either random hexamer or oligo(dT) primers (Superscript Preamplification System, Gibco BRL). A region corresponding to exons 39 and 47 of the

COL11A2 gene and exons 35 to 41 of the *COL2A1* gene was amplified by PCR from the cDNAs of individual II:2 and proband J35, respectively. For the RT-PCR analysis of *COMP* in MED patients 7 and 9, primers were designed to amplify the *COMP* cDNA in five overlapping fragments. PCR amplifications of the cDNAs were performed with gene-specific primers using AmpliTaq Gold DNA polymerase (Applied Biosystems), and these products were analyzed on agarose gels. The PCR products of the *COL11A2* cDNA were analyzed on agarose gels, cloned, and sequenced; for the other genes the obtained PCR products were directly sequenced with RT-PCR or nested sequencing primers as described in section 4.3.

4.5 Genotyping of polymorphisms for OA association analysis (I)

Six to twelve relatively common polymorphisms that were initially detected as heteroduplexes in CSGE analysis were selected from each cartilage collagen gene for full genotyping in 72 Finnish OA cases and 103 controls. This lead to the analysis of a total of 49 single nucleotide polymorphism (SNPs) and other polymorphic sites. These polymorphisms were analyzed either by direct sequencing of PCR products or by restriction fragment length polymorphism analysis of PCR products as indicated in the supplemental information to article I.

4.6 Linkage analysis of candidate genes in MED families 7 and 9 (III)

The known loci for autosomal dominant MED (*COL9A1*, *COL9A2*, *COL9A3*, *COMP* and *MATN3*) were analyzed for linkage in the families of MED patients 7 and 9. Markers 509-8B2 (in intron 5 of *COL9A1*) and 509-12B1 (<15 kb 5' of *COL9A1*) were used for *COL9A1*, the ATTA/GT repeat (<29 kb 5' of COL9A2) and L-myc (200 kb 5' of *COL9A2*) for *COL9A2,* the CA repeat (16 kb 5' of *COL9A3*) for *COL9A3*, D19S460 (450 kb 5' of *COMP*), the TAAA repeat (in intron 9 of *COMP*) and D19S443 (225 kb 3' of *COMP*) for *COMP,* and the GT repeat (in intron 3 of *MATN3*) for *MATN3* and analyzed as previously described (Mortier *et al.* 2001, Briggs & Chapman 2002). In addition, three *COMP* polymorphisms, c.1755G>A, c.1915-45C>T, and c.2228-40T>C, were genotyped in both families by sequencing.

4.7 Statistical analysis (I)

The computer program Polyphen was used to predict whether observed amino acid substitutions were likely to be pathogenetic (Sunyaev *et al.* 2000, Sunyaev *et al.* 2001, Ramensky *et al.* 2002). The observed allele frequencies for all the genotyped polymorphisms were compared between the OA cases and controls. To verify that the allele frequencies do not differ between the individuals enrolled at Jyvaskylä Central Hospital and Kuopio University Hospital, negative control analyses were conducted

between cases from both hospitals and between controls from both hospitals. Fisher's "exact" test was used for the analysis of individual diallelic polymorphisms, one polymorphism at a time. Stratification of data by gender and the affected joint was performed and the observed allele frequencies in these subgroups were compared to those of the control group using Fisher's "exact" test. Joint analysis of all polymorphisms within a gene was performed using the Dismult computer program, which implements a multiple two-point approach for linkage disequilibrium analysis (Terwilliger 1995).

5 Results

5.1 Mutation and association analysis of cartilage collagen genes in patients with primary early-onset hip and/or knee OA (I)

The screening of cartilage collagen genes (*COL2A1, COL9A1, COL9A2, COL9A3, COL11A1,* and *COL11A2*) in 72 Finnish probands with early-onset hip and/or knee OA and in a North American family with early-onset OA by CSGE and sequencing identified altogether 239 sequence variations in probands; sixteen of them were not found in controls. Two of these unique variants were in *COL2A1*, five in *COL11A1*, three in *COL11A2*, two in *COL9A*1, one in *COL9A2,* and three in *COL9A3* (Table II in article I). Seven of the variations, one in *COL2A1*, four in *COL11A1,* and two in *COL11A2*, were studied in more detail because they were predicted to result in amino acid substitutions or cause splicing abnormalities. In addition, the association of polymorphisms in cartilage collagen genes with primary early-onset hip and/or knee OA was evaluated by genotyping from six to twelve common polymorphisms from each gene in 72 OA patients and 103 controls and comparing the observed allele frequencies between these groups.

5.1.1 Identification of mutations in cartilage collagen genes in OA patients with early-onset hip and/or knee OA

A small US family with early-onset OA was referred to us for mutation analysis (Figure 1 in article I). The proband had undergone bilateral hip and knee replacements due to severe OA. She had near-normal vision, but had developed hearing loss with advancing age. Her daughter was asymptomatic, but her son was found to have some very mild Stickler-like features in addition to OA of the knees and cervical spine. Analysis of *COL11A2* identified the deletion of nucleotide A (c.3151-2) at the 3'-splice site of intron 42 (data not shown) in both affected family members, and RT-PCR analysis of RNA prepared from lymphocytes of the proband's affected son was performed to study the

effect of this variation on splicing. Two PCR products were obtained, one of about 750 bp corresponding to the wild-type allele and one of about 650 bp. Sequencing indicated that the shorter one was lacking the sequences for exon 43, and thus had an in-frame deletion of 108 bp, or 36 amino acids.

Seventy-two Finnish probands with primary early-onset hip and/or knee OA were screened by CSGE for sequence variants in the six genes coding for collagens II, IX and XI. Fifteen unique variants not present in controls were identified. Two of these unique variants were in *COL2A1*, five in *COL11A1*, two in *COL11A2*, two in *COL9A*1, one in *COL9A2,* and three in *COL9A3*. Six of the variations, found in five probands, were studied in more detail because they were predicted to result in either splicing defects or substitutions of conserved amino acids (Table II in article I). To further ascertain the role of these sequence variations in OA, members of the families of four of the five probands were examined. Co-segregation of the sequence variation and the phenotype was observed in three of these families, while the fourth could not be assessed because the parents of the proband were deceased and the only close relative available for testing was the proband's unaffected sister, who did not have these variants. One individual in the random population was found to have the two variations, however, and clinical and radiological examinations revealed that she also had OA, suggesting some significance of these variations for the disease. One of the variations, a c.2659G>A change in the last nucleotide of exon 38 of *COL2A1,* was shown to be likely to cause a quantitative or "leaky" splicing defect that results in aberrant splicing and nonsense-mediated mRNA decay, most likely by the use of a cryptic splice site in some but not all mRNAs from the mutant allele. None of the unique sequence variations identified in the collagen IX genes altered the known splicing consensus sequences or resulted in amino acid substitutions.

5.1.2 No evidence for the association of polymorphisms in cartilage collagens with primary early-onset hip and/or knee OA

Between six and twelve polymorphisms were chosen, on the basis of being relatively common, for full genotyping in all six examined collagen genes, leading to a total of 49 SNPs and other polymorphic sites being studied. The observed allele frequencies were compared between the 72 unrelated Finnish probands and the 103 unrelated Finnish controls. Fisher's "exact" test was used to analyze the individual diallelic polymorphisms, one polymorphism at a time. No statistically significant differences in allele frequencies (after adjusting for multiple testing) were detected between the probands and controls at any site in any gene, either when considering all the affected subjects (Table 3 in article I) or when subdividing them into cases of OA of the knee or the hip (data not shown). Stratification of the data set by sex showed a marginal difference in the allele frequencies of two SNPs (c.3025-23A>T, p 0.028; c.4512C>T, p 0.033) in *COL11A1* between the female cases and controls, but when multiple testing was taken into account these differences were no longer considered significant.

5.2 Identification of mutations in MED patients and the clinical and radiographical analysis of MED patients (II, III)

5.2.1 Identification of a recurrent R718W mutation in COMP and the description of the associated MED phenotype (II)

A three-generation MED family where two children cases presented with muscular weakness, moderate creatine kinase elevation, and knee joint epiphyseal dysplasia was clinically and radiographically examined and then referred to us for mutation analysis (Figure 1, family 1 in article II). Clinical and radiographic findings suggested a collagen IX mutation (Figures 2 and 3 in article II), but screening of the candidate exons of *COL9A1*, *COL9A2,* and *COL9A3* by CSGE and sequencing yielded negative results. Instead, a heterozygous R718W mutation in the *COMP* gene was identified (Figure 5 in article II), which was the same mutation that had recently been reported in a Japanese MED patient (Mabuchi *et al.* 2003). The same mutation was then identified in a second family with dominantly inherited MED (family 2 in Figure 1 in article II) that had similar radiographic findings and also had tested negative for collagen IX mutations. Even though the radiographical findings of these patients were similar to those reported in patients with collagen IX mutations, the affected family members in the first family developed early-onset osteoarthritis of the hips requiring THR when the patients were in their 30's or 40's.

Analysis of radiographic changes in the knee joints of these seven individuals (Figure 3 in article II) with the same *COMP* mutation illustrated the time window in which epiphyseal changes were recognizable and their dynamic nature. Between age 11 yrs and 13 yrs (probably coincidental with the pubertal growth spurt and osseous maturation), an additional ossification appeared in the medial condyles of the femur and tibia, and extended to form a ring at the periphery of the epiphysis. The finding was characteristic at this stage (resembling a "crevice" in a glacier) at this age and could be seen both in the distal femoral and in the proximal tibial epiphysis. Before this age period, epiphyses were moderately small with irregular contours. At later ages, the "crevice" sign was no longer visible; the epiphyses were flatter than normal.

5.2.2 Results of the mutation screening of COL9A1, COL9A2, COL9A3, COMP, DTDST, and MATN3 in the Canadian MED patient cohort (III)

Altogether 29 consecutive unrelated MED patients, who were followed at the Orthopedic Clinic, The Hospital for Sick Children, were included in the study. Detailed clinical and mutation data had previously been reported for four of these patients: three patients with clinical findings consistent of MED were homozygous for *DTDST* mutations (Ballhausen *et al.* 2003, Mäkitie *et al.* 2003) and one patient had findings typical of EDM1 and had a

COMP mutation (Susic *et al.* 1997). Mutation screening of the known MED genes (*COL9A1*, *COL9A2*, *COL9A3*, *COMP*, *DTDST* and *MATN3)* in the remaining 25 patients or their affected parents was performed using CSGE and/or direct sequencing, and the causative mutation was identified in six of the 25 patients (Table 1 in article III).

Two patients were found to have a *COMP* mutation, one was homozygous for the most common rMED mutation (R279W) in *DTDST,* and three patients had a *MATN3* mutation. MED patient 20 with a *de novo* D422A mutation in the 6th T3-repeat in COMP had clinical and radiographical findings consistent with EDM1, whereas MED patient 10 with an A735V mutation in the C-terminus of COMP had myopathic symptoms in addition to epiphysel dysplasia. This patient had inherited the mutation from her mother, who had had knee and hip pain since her teens and had undergone bilateral hip THR in adulthood. Patient 19 had a clubfoot deformity in addition to generalized epiphyseal dysplasia and he was found to be homozygous for the most common rMED mutation R279W in *DTDST*. Two mutations in *MATN3* were identified in three probands: a recurrent R^{121}W mutation in MED patient 1 and a recurrent I192N mutation in MED patients 4 and 13 (Table 1 in article III, Jackson *et al.* 2004). Their clinical and radiographical findings were very similar to those described in *MATN3*-associated MED. No disease-causing mutations were found in the CSGE and sequencing analyses of exons 8-10 of *COL9A1* or exons 2-4 of *COL9A2* and *COL9A3*.

The mutation was identified in altogether 10 of the 29 probands, including the previously reported mutations (Susic *et al.* 1997, Ballhausen *et al.* 2003, Mäkitie *et al.* 2003). Four patients had a mutation in *DTDST* (14 %), three in *COMP* (10 %), and three (10 %) in *MATN3*; no mutations were identified in the collagen IX genes. Thus, the known MED genes were responsible for 34 % of the cases in this cohort.

5.2.3 Linkage analysis of candidate genes in the families of Canadian MED patients 7 and 9 and the results of RT-PCR analysis of COMP in these patients (III)

Since the phenotype of MED in the families of patients 7 and 9 was typical for MED and the mutation screening of *COMP*, *COL9A1*, *COL9A2*, *COL9A3*, *DTDST,* and *MATN3* at the DNA level had yielded negative results, we wanted to study whether we could exclude these known candidate genes. Clinical data and blood samples were obtained from family members (Figure 1a and 1b in article III), and linkage analysis with microsatellite markers for *COL9A1*, *COL9A2*, *COL9A3*, *COMP,* and *MATN3* was performed. Recombinations were observed between *COL9A1*, *COL9A3*, *COL9A3,* and *COMP* and the MED phenotype in family 7, the loci for *MATN3* could not be excluded since all family members were homozygous for the *MATN3* marker. The loci for *COL9A1* and *COMP* could not be excluded in family 9. (Figure 1a and b in article III).

RNA analysis of *COMP* in patients 7 and 9 was performed to search for mutations possibly resulting in larger gene rearrangements and to confirm the negative findings of the mutation screening of *COMP* at the DNA level, since initially the *COMP* locus could not be excluded in the linkage analysis. Also, the mutation screening of *COMP* by CSGE

and sequencing did not identify a disease-causing mutation. The *COMP* cDNA was amplified in five overlapping fragments and no size differences or sequence variations were detected in the PCR-amplified cDNA fragments of patients 7 and 9.

5.2.4 Identification of two new phenotypic entities among patients with no mutations in the known MED genes (III)

No disease-causing mutation in the known MED genes was identified in 19 of the 29 patients. In all, 18 of them were found to have variable bilateral hip involvement when their hip radiographs were analyzed. Knee radiographs were available for 16 of the patients, and all except one had radiographic changes in the knees. Hand radiographs were usually normal. Seven patients were found to have mild spinal changes, and ankle radiographs revealed epiphyseal changes in seven cases. (Table 1 in article III).

Two distinctive new phenotypic entities were identified among the patients with no observed mutations. One of them was characterized by severe, early-onset dysplasia of the proximal femurs with almost complete absence of the secondary ossification centers, and abnormal development of the femoral necks was found in three of the MED patients (patients 17, 24, and 25 in Table 1 in article III, Figures 3 and 4 in article III). The other phenotype was characterized by “mini-epiphyses”, resulting in severe dysplasia of the proximal femoral heads (Figures 5 and 6 in article III) in two patients (patients 14 and 16 in Table 1 in article III) with no family history of MED, suggesting either a new dominant mutation or recessive inheritance.

6 Discussion

6.1 The role of genetic factors in osteoarthritis

Over the past decade, about 1200 genes causing human diseases and traits have been identified, largely by a process that is generally referred to as 'positional cloning'. This approach has been fruitful in Mendelian diseases, identifying high relative risk genes in these diseases, where the observed phenotype was due to a simple difference in a single gene or locus that usually caused a rare and severe early-onset phenotype (Risch 2000, Peltonen & McKusick 2001, Botstein & Risch 2003). However, the identification of genes involved in more common complex chronic diseases has been a major challenge to the scientific community. These diseases typically vary in the severity of symptoms and age of onset, resulting in difficulies in defining an appropriate phenotype and selecting the best population to study. In addition, they can vary in their etiological mechanisms, possibly involving various biological pathways. But perhaps most importantly, these complex diseases are more likely to be caused by mutations in several, and even numerous genes, each with a small overall contribution and relative risk (Risch 2000, Peltonen & McKusick 2001, Tabor *et al.* 2002, Botstein & Risch 2003).

Osteoarthritis is an example of a chronic disease with a multifactorial etiology that includes modifiable risk factors such as obesity, in addition to a strong genetic susceptibility (Felson & Zhang 1998, Felson *et al.* 2000). Classic twin studies have shown strong heritability for hand (56-65 %) and hip OA (60 %) and for disc degeneration of the cervical and lumbar spine (73- 75 %), whereas the heritability for knee OA is lower (39 %- 62 %) (Spector & MacGregor 2004, for further review see section 2.5.4). These and the siblings risk studies that have been performed have highlighted potential differences in the degree of OA heritability between different joint sites and between sexes, suggesting a high level of heterogeneity in the nature of the encoded susceptibility (Loughlin 2003). The finding that OA susceptibility is largely determined by genetic factors had prompted the search for OA genes. Clues for OA candidate genes have come from studies of inherited chondrodysplasias in which OA forms a part of the phenotype (for reviews, see Kuivaniemi *et al.* 1997, Baitner *et al.* 2000, Reginato & Olsen 2002), from mouse models of OA (for a review, see Helminen *et*

al. 2002) and from rare examples of familial OA, which have tended to implicate the gene for collagen II encoding the major collagen of cartilage (see section 2.4.1.1. and Spector & MacGregor 2004).

There is evidence for the possible involvement of *COL2A1* sequence variations in OA susceptibility based on the rare familial OA cases with *COL2A1* mutations (Ala-Kokko *et al.* 1990, Williams *et al.* 1993, Pun *et al.* 1994, Bleasel *et al.* 1995, Williams *et al.* 1995, Bleasel *et al.* 1996, Holderbaum *et al.* 1996, Bleasel *et al.* 1998, Moskowitz *et al.* 2004, Löppönen *et al.* 2004), and an increased tendency to develop OA in several heterozygous transgenic mouse lines (for a review, see Helminen *et al.* 2002). Association studies have also provided some evidence for the role of *COL2A1* polymorphisms in OA (for a review, see Loughlin 2001). Similarly, mutations in the collagen IX genes have been shown to cause MED, which is a relatively mild chondrodysplasia characterized mainly by knee OA in adult patients (see section 2.6.2.2.). Further support for the possible role of collagen IX in OA had been obtained from animal studies in which mice lacking the α1(IX) chains (Fässler *et al.* 1994) and transgenic mice heterozygous for a large in-frame deletion of *Col9a1* (Nakata *et al.* 1993, Kimura *et al.* 1996) developed degenerative joint disease similar to human OA. Mutations in the *COL11A1* and *COL11A2* genes have been shown to cause different chondrodysplasias, including Stickler and Marshall syndromes, and recessive otospondylomegaepihyseal dysplasia (for reviews, see Baitner *et al.* 2000 and Reginato & Olsen 2002), in which precocious OA is typically present. Two recent studies indicated that heterozygosity for a loss-of-function mutation in *Col11a1* resulted in the increased development of OA in heterozygous cho/+ mice (Xu *et al.* 2003, Rodriquez *et al.* 2004).

With this indirect evidence suggesting a potential role for these cartilage collagen genes in OA, analyses of the *COL2A1*, *COL9A1*, *COL9A2*, *COL9A3*, *COL11A1*, and *COL11A2* genes in 72 unrelated Finnish OA probands with early-onset primary hip and/or knee OA and in a North American family were performed using CSGE and sequencing to study whether there were rare sequence variants in these genes that would contribute to OA susceptibility as rare high-risk alleles. A subsequent association analysis using 6 to 12 simple nucleotide polymorphisms including SNPs and small deletion/insertion polymorphisms from each gene, leading to a total of 49 polymorphisms being analyzed, was performed to study whether these more common variants contributed to OA susceptibility as moderate-to-low risk alleles.

Although no common predisposing alleles were found in any of the genes in the association analysis, the screening for sequence variants identified altogether 16 unique sequence variations in the cartilage collagen genes in thirteen probands. Seven of the variations, found in six probands, were studied in more detail because they were predicted to result in either splicing defects or substitutions of conserved amino acids. To further ascertain the role of the sequence variations in OA, members of the families of five of the six probands were examined. Co-segregation of the sequence variation and the phenotype was observed in four of these families, while the fifth could not be assessed because the parents of the proband were deceased and the only close relative available for testing was proband's unaffected sister, who did not have these variants. As in other complex diseases, these families with potential disease-causing variants may represent the rare Mendelian, early-onset form of OA. To estimate the proportion of OA cases due

to these observed variants or the risk they cause requires study using larger patient and control material.

The finding of certain types of mutations, including the G to A change in the last nucleotide of exon 38 of *COL2A1* resulting in a splicing defect and nonsense-mediated mRNA decay, as well as the splicing mutation c.3151-2delA in intron 42 of *COL11A2* which results in the skipping of exon 43 and thus an in-frame deletion of 108 bp, that have usually been associated with chondrodysplasias (Vikkula *et al.* 1995, Sirko-Osadsa *et al.* 1998, Reginato & Olsen 2002, Vuoristo *et al.* 2004) suggest the possibility that some of the OA patients with early-onset OA may have a mild form of chondrodysplasia that presents only as early-onset OA later in life. The significance of the identified *COL2A1* mutation that possibly leads to the slightly reduced expression but not haploinsuffiency of *COL2A1* in OA pathogenesis is supported by the report of Loughlin *et al.* (1995), who showed that the reduced expression of one of the *COL2A1* alleles can contribute to the development of OA. Thus, it is possible that a minor reduction in *COL2A1* expression due to different mutations could result in the OA phenotype, whereas the haploinsufficiency of *COL2A1* results in Stickler syndrome (van der Hout *et al*. 2002)

Interestingly, four missense mutations affecting conserved amino acids were found in *COL11A1* and one in *COL11A2* in OA patients. The identified R53W mutation in the α2(XI) chain is of particular interest, since this variant co-segregated with the OA phenotype in the family. Tryptophan is not normally present in the triple-helical domains of collagens, and two Trp mutations in collagen IX have previously been reported to be associated with a common human disease, lumbar disc disease (Annunen *et al.* 1999a, Paassilta *et al.* 2001). It is possible that tryptophan, the most hydrophobic amino acid, has an effect on the triple-helix conformation or interactions with other matrix molecules. Two recent independent analyses of the cho/+ mouse model, where mice are heterozygous for a loss-of-function mutation in *Col11a1,* have shown development of OA in these otherwise normal mice with increased degradation of collagen II similar to that seen in human OA (Xu *et al.* 2003, Rodriguez *et al.* 2004). Taken together, these and our results suggest that mutations in the genes encoding the collagen α1(XI) and α2(XI) chains can cause increased susceptibility to OA in a subgroup of OA patients. Whether these mutations have a dominant-negative effect or possibly lead to premature degradation of mutant α-chains remains to be studied.

No statistically significant differences were detected in allele frequencies (after adjusting for multiple testing) between the probands and controls at any site in any gene in our association analysis, either when considering all the affected subjects or when subdividing them into cases of OA of the knee or the hip, or when subdividing cases by sex. However, our sample size was quite small, decreasing the power to detect common, low-risk alleles. Also, partly due to the small sample size, we did not attempt to estimate haplotype frequencies for all the polymorphisms genotyped in a given collagen gene, nor compare them between the probands and controls even though the use of haplotypes over single SNPs has been more powerful in many cases (Cardon & Bell 2001). In addition, our controls were not radiographically examined, though they were free of clinical OA, the sex and age distribution was similar to OA cases, and no population stratification due to different ethnicity was present as both groups were from Central Finland. Results from previous association studies of *COL2A1* and OA have been somewhat contradictory (Loughlin 2001). No evidence of association was found between *COL9A1* with hip OA in

a British study (Loughlin *et al.* 2002c). A Japanese group studied the association of a few SNPs from each cartilage collagen gene and found modest evidence for the association of female knee OA with a synonymous c.1740C>T SNP in *COL9A3* (Ikeda *et al.* 2002), but this SNP showed no association in our study. Thus, our results suggest, and are supported by the views of Reginato and Olsen (Reginato & Olsen 2002), that OA may consist of a group of distinct but overlapping phenotypes, and that the number of genes and alleles within a gene involved in its pathogenesis may well be higher than is currently thought.

The findings of OA association studies have generally been inconsistent, with the most consistent findings being for the involvement of *VDR* and *IL-1* gene cluster variants as OA susceptibility loci (Keen *et al.* 1997, Uitterlinden *et al.* 1997, Uitterlinden 2000, Loughlin *et al.* 2000, Meulenbelt *et al.* 2004, Smith *et al.* 2004; for a review see, Spector & Macgregor 2004). Some of the studies have used radiological evaluation of population based samples (The Rotterdam cohort, Chingford cohort), whereas most of studies performed to date are case-control studies, in which OA cases usually have had severe end-stage OA as ascertained by the need for total joint replacement. Controls used in these studies have usually been asymptomatic for joint problems, but their disease status has rarely been ascertained using radiographic examination. In addition, most of the association studies with case-control design have been of limited sample size, leading to a decrease in statistical power to detect small effects and thereby increasing the probability of false-positive findings. Furthermore, not all studies have adjusted the findings for potential confounders, such as age, gender, and BMI. In larger studies with positive findings, the phenotype has usually been specific, involving only hand, hip or knee OA, not a mixture of all of these. As proposed by Tabor *et al.* (2002) and Risch (2000) in general, the non-replication seen in OA association studies is likely to be due to differences in the study design and from a low prior probability that the few gene polymorphisms examined are in fact causally related to the disease outcomes studied, even though population stratification might also play a role.

The studies assessing the role of genetics in OA have shown that better characterization of the OA phenotype would increase the possibility for positive findings both in association and linkage analyses. The heterogeneity in the phenotype is known to decrease the ability to detect linkage (Altmüller *et al.* 2001, Botstein & Risch 2003), and this has been shown by OA linkage studies, where the site-specificity of the gene effects has been demonstrated. This is shown by the findings of Loughlin *et al.* (1999), who after stratification their data into hip and knee OA were able to detect multiple suggestive linkages. Further support came from the linkage analysis of hand OA in the Framingham cohort, where the initial analysis of hand OA as a single entity showed only a suggestive linkage to various chromosomal regions, whereas after joint-specific analysis, the obtained results markedly improved with significant linkage of CMC1 OA to chromosome 15 with multipoint LOD score (MLS) 6.25 being found (Demissie *et al.* 2002, Hunter *et al.* 2004). In addition, association studies have implicated different genes (e.g. *COL2A1* and *VDR*) with the occurrence of jointspace narrowing and osteophytosis (Uitterlinden *et al.* 2000, Valdes *et al*. 2004). Thus, it is clear that OA studies will provide more information if careful assessment of the selected OA phenotype is performed (Spector & MacGregor *et al.* 2004).

The results of OA linkage studies have rarely been replicated, and this has also been the case with OA association studies (for a review, see Loughlin *et al.* 2003). For hip OA,

linkage to overlapping regions of chromosome 16 has been reported by two different studies (Loughlin *et al.* 1999, Ingvarsson *et al.* 2001, Forster *et al.* 2004), whereas linkage to chromosomes 2q24.3-q31.1, 4q13.1-4q13.2, 6p12.3-q13, 11q13.4-q14.3, and 11q21.1-q23.2 has not been replicated (Chapman *et al.* 1999, Loughlin *et al.* 1999, 2000, Chapman *et al.* 2002, Loughlin *et al.* 2002c,d; Loughlin 2003, Forster *et al.* 2004b, Loughlin *et al.* 2004). However, evidence of the linkage of hand OA to the chromosome 11q region overlaps with the linkage of hip OA to 11q13.4-q14.3, suggesting that 11q is likely to harbor an OA susceptibility gene(s) (Demissie *et al.* 2002, Chapman *et al.* 2002). The most promising linkage of hand OA has been to the chromosome 2p24-p21 region detected by two large independent linkage studies (Demissie *et al.* 2002, Stefansson *et al.* 2002). One of these studies reported the association of hand OA with the T303M variant in *MATN3* (Stefansson *et al.* 2003). In addition, various other chromosomal regions warrant further study (Leppävuori *et al.* 1999, Demissie *et al.* 2002, Stefansson *et al.* 2003, Hunter *et al.* 2004). (For reviews, see Loughlin 2002, Loughlin 2003, and Spector & MacGregor 2004.) In our initial findings of the genome-wide linkage analysis, a cohort of 10 Finnish families with hip and/or knee OA showed a suggestive linkage to chromosome 2q21-q23 with a multipoint lod score of 2.135 occurring between markers D2S112 (141 cM) and D2S142 (161 cM) and to a region around 11q23 with a maximum multipoint lod score of 1.824 at marker D11S925 (123.5-126 cM). These results support the findings of a UK group even though the regions are not completely overlapping (Jakkula *et al.*, unpublished data). Fine mapping and association analysis of the above-mentioned chromosomal regions is likely to define multiple moderate-to-low OA risk alleles, whereas some of the OA families are likely to have high-risk alleles in other genes. The search for these genes goes on, and hopefully positive results from these studies combined with a wealth of new information from functional genomics and cell and animal model studies of OA will enable the development of new means for diagnosis, follow-up, and treatment of OA patients.

6.2 Allelic and non-allelic heterogeneity in MED in relation to genotype-phenotype correlations

As the identification of genes responsible for Mendelian disorders has been much more successful than in complex diseases, the genetic basis of MED has also been partially solved during the last decade. MED is a clinically heterogenic disorder with variable degrees of severity and with variable radiological findings. Common to all forms is the generalized epiphyseal dysplasia affecting mainly the hips and knees, whereas the absence of severe spinal involvement and minimal metaphyseal defects allow the differential diagnosis of MED from other chondrodysplasias with similar clinical features (e.g. spondyloepimetaphyseal dysplasia and spondyloepiphyseal dysplasia). MED is also genetically heterogenous, showing both allelic and non-allelic genetic heterogeneity, as causative mutations have been identified in five different genes (*COMP*, *COL9A1*, *COL9A2*, *COL9A3*, and *MATN3*) in the dominant form of MED and in one gene (*DTDST*) in the recessive form. In addition to this genetic heterogeneity, there is marked

intrafamilial variability in clinical phenotype, suggesting that environmental or modifying genetic factors also play a role. (For a recent review, see Chapman *et al.* 2003).

To further characterize the genotype-phenotype correlations in MED, a clinical, radiographical, and mutation analysis of two families with MED suggesting a collagen IX mutation and of a cohort of 29 unrelated Canadian MED cases was performed. Radiographic features and the presence of myopathy in the first MED family suggested a collagen IX mutation, but screening of *COL9A1*, *COL9A2,* and *COL9A3* yielded negative results. Instead, a heterozygous R718W mutation in the *COMP* gene was identified. The same mutation was then identified in a second family with dominantly inherited MED that had similar radiographic changes and also had tested negative for collagen IX mutations. Mabuchi *et al.* (2003) had previously observed the same R718W mutation segregating within a family, but little clinical details or radiographic data were initially given, except for height at –3 SD and a diagnosis of "Fairbank" type MED. After our report, they reported that in their affected family members, the hips were the most severely affected and myopathy was not present. Their cases with the *COMP* R718W mutation were evaluated in adulthood, and thus it is difficult to compare the phenotype in detail, but in both families hip changes were progressive leading to a need for total hip replacement (Igekawa 2004). The knee "crevice" sign and the accessory cranial ossification center of the patella seen in the patients with a *COMP* R718W mutation have also been observed in collagen IX-associated MED (Muragaki *et al.* 1996, Lohiniva *et al.* 2000). Thus, neither of the signs is specific for a collagen IX mutation and there is a considerable overlap in phenotypes caused by mutations in different MED genes. Unlike collagen IX mutations, this COMP R718W mutation led to early-onset hip OA requiring THR in early adulthood which is not usually seen in collagen IX-associated MED.

The analysis of *COL9A1*, *COL9A2*, *COL9A3*, *COMP*, *DTDST,* and *MATN3* in the Canadian MED cohort of 29 probands identified a disease-causing mutation in 10 probands (34 %); the detailed results for four of the probands had been reported previously (Susic *et al.* 1997, Ballhausen *et al.* 2003, Mäkitie *et al.* 2003). Of the 25 unrelated probands analyzed here, three had a *MATN3* mutation, two had a *COMP* mutation, and one proband was homozygous for the most common rMED mutation, $R^{279}W$ in *DTDST*. When the previously reported results are combined with the ones from this study, a *DTDST* mutation was found in four probands (14 %), a *COMP* mutation in three (10 %), and a *MATN3* mutation also in three (10 %). No mutations were identified in collagen IX genes. The overall mutation frequency obtained in this study does not greatly differ from that reported in two slightly larger studies (Czarny-Ratajzak *et al.* 2001, Mabuchi *et al.* 2004), even though *MATN3* (24 %) and *COMP* (20 %) mutations have been found more frequently in the Japanese MED patients (Mabuchi *et al.* 2004).

When the results from the MED studies presented in this thesis are taken into account with all previously reported studies, altogether 47 different MED causing mutations have been identified in 82 probands, with nine mutations being recurrent. *COMP* and *DTDST* mutations seem to be the most common causes of MED cases with an identified genetic defect, as 24 different *COMP* mutations have been identified in 29 probands (35 % of the probands with an identified genetic defect) and five different *DTDST* mutations have been found either as homozygous or compound heterozygous in 25 probands (30 %) (Table 5). However, the causative mutation has been found in the known MED genes only in approximately 1/3-1/2 of the probands studied (Briggs *et al.* 1995, 1998; Czarny-

Ratajzak *et al.* 2001, Mabuchi *et al.* 2003, 2004, article III), suggesting that additional MED loci are likely to exist.

Table 5. Summary of identified mutations in MED probands by gene

Gene	Identified mutations	Recurrent mutations	Probands with identified mutation	% of probands
COMP	24	4	29	35[a]
COL IX	9	1	10	12[b]
MATN3	9	2	18	22[c]
DTDST	5*	2	25	30[d]
Total	46	9	82	

* Five different mutations in *DTDST* have been reported in MED patients, the number of different combinations is currently 6 (homozygous and compound heterozygous)

[a] References: Briggs *et al.* 1995, Ballo *et al.* 1997, Susic *et al.* 1997, Briggs *et al.* 1998, Igekawa *et al.* 1998, Loughlin *et al.* 1998, Deere *et al.* 1998, Delot *et al.* 1999, Czarny-Ratajzak *et al.* 2001, Briggs *et al.* 2002, Mabuchi *et al.* 2003, Song *et al.* 2003, articles II and III; [b] References: Muragaki *et al.* 1996, Holden *et al.* 1999, Paassilta *et al.* 1999, Bönnemann *et al.* 2000, Lohiniva *et al.* 2000, Spayde *et al.* 2000, Czarny-Ratajzak *et al.* 2001, Fiedler *et al.* 2002, Nakashima *et al.* 2005; [c] References: Chapman *et al.* 2001, Mostert *et al.* 2003, Jackson *et al.* 2004, Mabuchi *et al.* 2004, article III; [d] References: Superti-Furga *et al.* 1999, Czarny-Ratajzak *et al.* 2001, Huber *et al.* 2001, Ballhausen *et al.* 2003, Mäkitie *et al.* 2003, article III

The patients with identified mutations in the *COMP, MATN3,* and *DTDST* genes in this MED study presented with clinical and radiographical findings that were well in accordance with the phenotypes previously reported in the presence of similar mutations. It might be of interest to note that the proband with an A735V mutation in the C-terminus of COMP suffered from myopathic symptoms. Thus, the association of myopathic symptoms has been reported with two mutations in the C-terminus of *COMP* (R718W, A735V; articles II and III) and with two *COL9A3* mutations (Bönnemann *et al.* 2000, Lohiniva *et al.* 2000). Little is known so far about possible functions of collagen IX and COMP in muscle, and this aspect remains to be investigated. However, in a view of the molecular interactions between COMP and collagen IX (Holden *et al.* 2001, Pihlajamaa *et al.* 2004, Mann *et al.* 2004, Hecht *et al.* 2005), it is possible that a common supramolecular complex in muscle (possibly at the junction between myocytes and adjacent matrix structures) is affected. A careful assessment of clinical and radiographic features could point to a certain candidate gene to be analyzed first if molecular verification of MED is needed, even though there is a considerable overlap between phenotypes caused by *COMP*, the collagen IX genes and *MATN3* mutations (Mortier *et al.* 2001, Unger *et al.* 2001, Mäkitie *et al.* 2004, article II). The presence of clubfoot and/or double-layered patella is strongly suggestive for a *DTDST* mutation (Superti-Furga *et al.* 1999, Ballhausen *et al.* 2003, Mäkitie *et al.* 2003), even though not totally specific (Nakashima *et al.* 2005, in press).

Interestingly, a review of the radiographs of the MED patients with no identified mutations revealed at least two new specific phenotypic entities. One of them was characterized by severe, early-onset dysplasia of the proximal femurs with almost complete absence of the secondary ossification centres and abnormal development of the

femoral necks. The other phenotype was characterized by “mini-epiphyses”, resulting in severe dysplasia of the proximal femoral heads. One of the patients with severe early-onset dysplasia of the proximal femurs had a family history consistent with autosomal dominant inheritance, but the pedigree was not large enough for a genome-wide scan. The latter phenotypic entity might be due to a new dominant mutation or recessive inheritance, since neither of the patients with “mini-epiphysis” had a family history of MED.

The cellular and molecular pathology of MED is not well understood. As MED is allelic to PSACH, and similar kinds of mutations in the calcium-binding T3 repeats and in the C-terminal domain of COMP have been observed in both disorders (Briggs & Chapman 2002), some evidence for the cellular and molecular pathology of MED can be derived from PSACH studies. Some of these mutations have been shown to disrupt calcium-binding and protein folding (Hecht *et al.* 1995, 1998; Chen *et al.* 2000, Hou *et al.* 2000, Maddox *et al.* 2000) and this improper folding and abnormal processing of COMP has resulted in intracellular retention of COMP in enlarged rER cisternae (Maddox *et al.* 1997, Delot *et al.* 1998, Hecht *et al.* 1998). A recent crystallography study of some of the T3-repeats and the C-terminus of structurally related thrombospondin-1 predicted that mutations in the C-terminal domain of COMP are likely to disrupt the tertiary structure of the T3-C-terminus assembly, and thus the normal function of the protein (Kvansakul *et al.* 2004). A hypothesis that PSACH and MED/EDM1 result from the accumulation of mutant COMP within the chondrocyte, selectively impairing the secretion of other matrix proteins and leading to downstream effects of abnormal ECM and chondrocyte death, had been earlier proposed by Hecht and colleagues (Hecht et al. 1998, 2001; Duke *et al.* 2003, Hashimoto *et al.* 2003, Hecht *et al.* 2004, Hecht *et al.* 2005). Their recent study, which focused on the effects of three different PSACH mutations in a 3-D cell culture model on the appearance and accumulation of cartilage proteins within chondrocytes in cartilage nodules, showed that these *COMP* mutations were associated with the accumulation of COMP, collagen IX, and matrilin-3 in the rER cisternae. The amount of COMP, collagen IX, and matrilin-3 was greatly decreased in the PSACH matrices and in the growth plate of PSACH patients, and as a result the normal organization of the ECM was disturbed as shown by the absence of organized collagen fibril bundles (Hecht *et al.* 2005). These findings suggest that COMP, collagen IX, and matrilin-3 together play an important role in matrix assembly. Thus, it seems likely that mutations in any of these genes can lead to similar consequences at the ultrastructural level of the ECM and thus cause a similar MED phenotype. Other matrix molecules may be involved in this supramolecular structure, as it seems that fibromodulin is also retained within the rER (Vranka *et al.* 2001). Identifying the other proteins involved may help to define still unknown MED genes.

The cellular pathology of MED due to collagen IX and *MATN3* mutations is much less known. Studies have shown that collagen IX interacts with COMP (Holden *et al.* 2001, Mann *et al.* 2004, Pihlajamaa *et al.* 2004), and COMP interacts with matrilins (Mann *et al.* 2004). However, a study of growth plate chondrocytes from a patient with a collagen IX mutation showed no intracellular accumulation bodies, and the collagen fibrils of cartilage seemed normal in electron microscopy (van Mourik *et al.* 1998).

As the phenotype of some MED patients is mild and can present as early-onset OA in adulthood with radiographic features being sometimes indistinguishable from that of

primary OA (Kawaji *et al.* 2002, Unger *et al.* 2001), it can be speculated that sequence variations in these MED-associated genes may play a role in OA pathogenesis. However, no such mutations were found in the collagen IX genes in our study of Finnish early-onset OA patients or in *COMP* (J Lohiniva, personal communication). The *MATN3* gene maps to 2p24-23, and a linkage to the region including this gene has been identified in two linkage studies of hand OA (Demissie *et al.* 2002, Stefansson *et al.* 2003). The latter study found a T303M polymorphism in *MATN3* to associate with hand OA (Stefansson *et al.* 2003). Thus, the understanding of the pathogenesis and genes involved in MED could also aid in elucidating the pathogenesis of a more common cartilage disorder, OA.

References

Abu-Hijleh G, Reid O & Scothorne RJ (1997) Cell death in the developing chick knee joint. I. Spatial and temporal patterns. Clin Anat 10:183-200.

Adams JC (2001) Thrombospondins: multifunctional regulators of cell interactions. Annu Rev Cell Dev Biol 17:25-51.

Aigner T & Stöve J (2003) Collagens – major component of the physiological cartilage matrix, major target of cartilage degeneration, major tool in cartilage repair. Adv Drug Deliv Rev 55:1569-1593.

Ala-Kokko L & Prockop DJ (1990) Completion of intron-exon structure of the gene for human type II procollagen (COL2A1): variations in the nucleotide sequences of the alleles from three chromosomes. Genomics 8:454-460.

Ala-Kokko L, Baldwin CT, Moskowitz RW & Prockop DJ (1990) 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 87:6565-6568.

Ala-Kokko L, Kvist AP, Metsäranta M, Kivirikko KI, de Crombrugghe B, Prockop DJ & Vuorio E (1995) Conservation of the sizes of 53 introns and over 100 intronic sequences for the binding of common transcription factors in the human and mouse genes for type II procollagen (*COL2A1*) Biochem J 308:923-929.

Altman RD (1995) The classification of osteoarthritis. J Rheumatol 22, supplement 43:42-43.

Altmüller J, Palmer LJ, Fischer G, Scherb H & Wjst M (2001) Genomewide scans of complex human diseases: true linkage is hard to find. Am J Hum Genet 69:936-950.

Andersen H & Bro-Rasmussen F (1961) Histochemical studies in the histogenesis of the joint in human fetuses with special reference to the development of joint cavities in the hand and foot. Am J Anat 108:111-122.

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

Annunen S, Körkkö J, Czarny M, Warman ML, Brunner HG, Kääriäinen H, Mulliken JB, Tranebjærg L, Brooks DG, Cox GF, Cruysberg JR, Curtis MA, Davenport SLH, Friedrich CA, Kaitila I, Krawczynski MR, Latos-Bielenska A, Mukai S, Olsen BR, Shinno N, Somer M, Vikkula M, Zlotogora J, Prockop DJ & Ala-Kokko L (1999b) Splicing mutations of 54 bp exons in the *COL11A1* gene cause Marshall syndrome but other mutations cause overlapping Marshall/Stickler phenotypes. Am J Hum Genet 1999;65:974-983.

Annunen S, Paassilta P, Lohiniva J, Perälä M, Pihlajamaa T, Karppinen J, Tervonen O, Kröger H, Lähde S, Vanharanta H, Ryhänen L, Göring HHH, Ott J, Prockop DJ & Ala-Kokko L (1999a) An allele of COL9A2 associated with intervertebral disc disases. Science 285:409-412.

Aravind L & Koonin EV (2000) The STAS domain – a link between anion transporters and antisigma-factor antagonists (letter) Curr Biol 10:R53-55.

Arita M, Li S-W, Kopen G, Adachi E, Jimenez SA & Fertala A (2002) Skeletal abnormalities and ultrastructural changes of cartilage in transgenic mice expressing a collagen II gene (COL2A1) with a Cys for Arg-α1-519 substitution. Osteoarthritis Cartilage 10:808-815,

Aromaa A & Koskinen S (eds) (2002) Health and functional capacity in Finland. Baseline results of the Health 2000 health examination survey. Publications of the National Public Health Institute B3/2002. Helsinki

Aszódi A, Bateman JF, Gustafsson E, Boot-Handford R & Fässler R (2000) Mammalian skeletogenesis and extracellular matrix. What can we learn from knock-out mice? Cell Struct Funct 25:73-84.

Baitner AC, Maurer SG, Gruen BS & Di Cesare PE (2000) The genetic basis of the osteochondrodysplasias. J Pediatr Orthop 20:594-605

Ballhausen D, Bonafe L, Terhal P, Unger SL, Bellus G, Classen M, Hamel BC, Spranger J, Zabel B, Cohn DH, Cole WG, Hecht JT & Superti-Furga A (2003) Recessive multiple epiphyseal dysplasia (rMED): phenotype delineation in eighteen homozygotes for DTDST mutation R279W. J Med Genet 40:65-71.

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

Ballock RT & O'Keefe RJ (2003) The biology of the growth plate. J Bone Joint Surg 85A:715-726.

Barbieri O, Astigiano S, Morini M, Tavella S, Schito A, Corsi A, Di Martino, Bianco P, Cancedda R & Garofalo S (2003) Depletion of cartilage collagen fibrils in mice carrying a dominant negative Col2a1 transgene affects chondrocyte differentiation. Am J Physiol Cell Physiol 285:C1504-1512.

Bateman JF (2001) The molecular genetics of inherited cartilage disease. Osteoarthritis Cartilage 9 Suppl A:S141-S149.

Belluoccio D & Trueb B (1997) Matrilin-3 from chicken cartilage. FEBS letters 415:212-216.

Belluoccio D, Schenker T, Baici A & Trueb B (1998) Characterization of human matrilin-3 (MATN3) Genomics 53:391-394.

Bernays A (1878) Die Entwicklungsgesichte des kniegelenkes des menschen, mit bemerkungen uber die gelenke im allegemeinen. Morph Jahrb 4:403-446.

Bijkerk C, Houwing-Duistermaat JJ, Valkenburg HA, Meulenbelt I, Hofman A, Breedveld FC, Pols HAP, van Duijn CM & Slagboom PE (1999) Heritabilities of radiologic osteoarthritis in peripheral joints and of disc degeneration of the spine. Arthritis Rheum 42:1729-1735.

Bleasel JF, Bisagni-Faure A, Holderbaum D, Vacher-Lavenu MC, Haqqi TM, Moskowitz RW & Menkes CJ (1995) Type II procollagen gene (*COL2A1*) mutation in exon 11 associated with spondyloepiphyseal dysplasia, tall stature and precocious osteoarthritis. J Rheumatol 22:255-261.

Bleasel JF, Holderbaum D, Brancolini V, Moskowitz RW, Considine EL, Prockop DJ, Devoto M & Williams C (1998) Five families with arginine 519-cysteine mutation in COL2A1: evidence for three distinct founders. Hum Mutat 12:172-6.

Bleasel JF, Holderbaum D, Mallock V, Haqqi TM, Williams HJ & Moskowitz RW (1996) Hereditary osteoarthritis with mild spondyloepiphyseal dysplasia--are there "hot spots" on *COL2A1*? J Rheumatol 23:1594-1598.

Botstein D & Risch N (2003) Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. Nat Genet 33 Suppl:228-237.

Brandi ML, Gennari L, Cerinic MM, Becherini L, Falchetti A, Masi L, Gennari C & Reginster J-Y (2001) Genetic markers of osteoarticular disorders: facts and hopes. Arthritis Res 3:270-280.

Brewton RG & Mayne R (1994) Heterotypic type II, IX and XI fibrils: comparison of vitreous and cartilage forms. In: Yurchenco PD, Birk DE & Mecham RP (eds) Extracellular matrix assembly and structure. Academic Press, San Diego, p129-170.

Briggs MD & Chapman KL (2002) Pseudoachondroplasia and multiple epiphyseal dysplasia: mutation review, molecular interactions, and genotype to phenotype correlations. Hum Mutat 19:465-478.

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 (1995) Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene. Nat Genet 10:330-336.

Briggs MD, Mortier GR, Cole WG, King LM, Golik SS, Bonaventure J, Nuytinck L, De Paepe A, Leroy JG, Biesecker L, Lipson M, Wilcox WR, Lachman RS, Rimoin DL, Knowlton RG & Cohn DH (1998) Diverse mutations in the gene for cartilage oligomeric matrix protein in the pseudoachondroplasia-multiple epiphyseal dysplasia disease spectrum. Am J Hum Genet 62:311-9.

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

Buckwalter JA, Saltzman C & Brown T (2004) The impact of osteoarthritis. Implications for research. Clin Orthop Rel Res 427 Suppl:S6-S15.

Bunning RA & Russell RGG (1989) The effect of tumor necrosis factor α and γ-interferon on the resorption of human articular cartilage and on the production of prostaglandin E and of casainase activity by human articular chondrocytes. Arthritis Rheum 32:780-784.

Bönnemann CG, Cox GF, Shapiro F, Wu JJ, Feener CA, Thompson TG, Anthony DC, Eyre DR, Darras BT & Kunkel LM (2000) A mutation in the alpha 3 chain of type IX collagen causes autosomal dominant multiple epiphyseal dysplasia with mild myopathy. Proc Natl Acad Sci USA 97:1212-1217.

Camenisch TD & McDonald JA (2000) Hyaluronan: is bigger better? Am J Respir Cell Mol Biol 23:431-433.

Campbell IK, Piccoli DS, Roberts MJ Muirden KD & Hamilton JA (1990). Effects of tumor necrosis factor α and β on resorption of human articular cartilage and production of plasminogen activator by human articular chondrocytes. Arthritis Rheum 33:542-552.

Cardon LR & Bell JI (2001) Association study designs for complex diseases. Nat Rev Genet 2:91-99.

Cauley JA, Kwoh K, Egeland G, Nevitt MC, Cooperstein L, Rohay J, Towers A & Gutai JP (1993) Serum sex hormones and severity of osteoarthritis of the hand. J Rheumatol 20:1170-1175.

Chadjichristos C, Ghayor C, Kypriotou M, Martin G, Renard E, Ala-Kokko L, Suske G, de Crombrugghe B, Pujol J-P & Galéra P (2003). Sp1 and Sp3 transcription factors mediate interleukin-1β down-regulation of human type II collagen gene expression in articular chondrocytes. J Biol Chem 278:39762-39772.

Chapman K, Mustafa Z, Dowling B, Southam L, Carr AJ & Loughlin J (2002) Finer linkage mapping of primary hip osteoarthritis susceptibility on chromosome 11q in a cohort of affected female sibling pairs. Arthritis Rheum 46:1780-1783.

Chapman K, Mustafa Z, Irven C, Carr AJ, Clipsham K, Smith A, Chitnavis J, Sinsheimer JS, Bloomfield VA, McCartney M, Cox O, Cardon LR, Sykes B & Loughlin J (1999) Osteoarthritis-susceptibility locus on chromosome 11q, detected by linkage. Am J Hum Genet 65:167-174.

Chapman KL, Briggs MD & Mortier GR (2003) Review: clinical variability and genetic heterogeneity in multiple epiphyseal dysplasia. Ped Pathol Mol Med 22:53-75.

Chapman KL, Mortier GR, Chapman K, Loughlin J, Grant ME & Briggs MD (2001) Mutations in the region encoding the von Willebrand factor A domain of matrilin-3 are associated with multiple epiphyseal dysplasia. Nat Genet 28:393-396.

Chen H, Deere M, Hecht JT & Lawler J (2000) Cartilage oligomeric matrix protein is a calcium-binding protein, and a mutation in its type 3 repeats causes conformational changes. J Biol Chem 275:26538-26544.

Chen Y, Sumiyoshi H, Oxfor JT, Yoshioka H, Ramirez F & Morris NP (2001) Cis-acting elements regulate alternative splicing of exons 6A, 6B and 8 of the α1(XI) collagen gene and contribute to the regional diversification of collagen XI matrices. Matrix Biology 20:589-599.

Chitnavis J, Sinsheimer JS, Clipsham K, Loughlin J, Sykes B, Burge PD & Carr AJ (1997) Genetic influences in end-stage osteoarthritis: sibling risks of hip and knee replacement for idiopathic osteoarthritis. J Bone Joint Surg Br 79:660-664.

Creamer P & Hochberg MC (1997) Osteoarthritis. Lancet 350:503-509.

Cremer MA, Rosloniec EF & Kang AH (1998) The cartilage collagens: a review of their structure, organization, and role in the pathogenesis of experimental arthritis in animals and in human rheumatic disease. J Mol Med 76:275-288.

Czarny-Ratajczak M, Lohiniva J, Rogala P, Kozlowski K, Perälä M, Carter L, Spector TD, Kolodziej L, Seppänen U, Glazar R, Krolewski J, Latos-Bielenska A & Ala-Kokko L (2001) A mutation in COL9A1 causes multiple epiphyseal dysplasia: further evidence for locus heterogeneity. Am J Hum Genet 69:969-980.

Dahlberg L, Billinghurst C, Manner P, Nelson F, Webb G, Ionescu M, Reiner A, Tanzer M, Zukor D, Chen J, van Wart HE &Poole AR (2000) Selective enhancement of collagenase-mediated cleavage of resident type II collagen in cultured osteoarthritic cartilage and arrest with a synthetic inhibitor that spares collagenase 1 (matrix metalloproteinase 1). Arthritis Rheum 43:673-682.

Dawson PA & Markovich D (2005) Pathogenetics of the human SLC26 transporters. Curr Med Chem 12:385-96.

Deák F, Wagener R, Kiss I & Paulsson M (1999) The matrilins: a novel family of oligomeric extracellular matrix proteins. Matrix Biology 18:55-64.

Deere M, Sanford T, Francomano CA, Daniels K & Hecht JT (1999) Identification of nine novel mutations in cartilage oligomeric matrix protein in patients with pseudoachondroplasia and multiple epiphyseal dysplasia. Am J Med Genet 85:486-490.

DeLise A, Fischer L & Tuan R (2000) Cellular interactions and signalling in cartilage development. Osteoarthritis Cartilage 8:309-334.

Delot E, Brodie SG, King LM, Wilcox WR & Cohn DH (1998) Physiological and pathological secretion of cartilage oligomeric matrix protein by cells in culture. J Biol Chem 273:26692-26697.

Delot E, King LM, Briggs MD, Wilcox WR & Cohn DH (1999) Trinucleotide expansion mutations in the cartilage oligomeric matrix protein (COMP) gene. Hum Mol Genet 8:123-128.

Demissie S, Cupples LA, Myers R, Aliabadi P, Levy D & Felson DT (2002) Genome scan for quantity of hand osteoarthritis. Arthritis Rheum 46:946-952.

DiCesare P, Hauser N, Lehman D, Pasumarti S & Paulsson M (1994b) Cartilage oligomeric matrix protein is an abundant component of tendon. FEBS letters 354:237-240.

DiCesare P, Mörgelin M, Mann K & Paulsson M (1994a) Cartilage oligomeric matrix protein and thrombospondin 1. Purification from articular cartilage, electron microscopic structure and chondrocyte binding. Eur J Biochem 223:927-937.

DiCesare PE, Chen FS, Mörgelin M, Carlson CS, Leslie MP, Perris R & Fang (2002) Matrix Biology 21:461-470.

Donahue LR, Chang B, Mohan S, Miyakoshi N, Wergedal JE, Baylink DJ, Hawes NL, Rosen CJ, Ward-Bailey P, Zheng QY, Bronson RT, Johnson KR & Davisson MT (2003) A missense mutation in the *Col2a1* gene causes spondyloepiphyseal dysplasia congenital, hearing loss and retinoschisis. J Bone Miner Res 18:1612-1621.

Doskocil M (1985) Formation of the femoropatellar part of the human knee joint. Folia Morph 33:38-47.

Duke J, Montufar-Solis D, Underwood S, Lalani Z & Hecht JT (2003) Apoptosis staining in cultured pseudoachondroplasia chondrocytes. Apoptosis 8:191-197.

Ekman S, Reinholt FP, Hultenby K & Heinegård D (1997) Ultrastructural immunolocalization of cartilage oligomeric matrix protein (COMP) in porcine growth cartilage. Calcif Tissue Int 60:547-553.

Everett LA, Morsli H, Wu DK & Green ED (1999) Expression pattern of the mouse ortholog of the Pendred's syndrome gene (Pds) suggests a key role for pendrin in the inner ear. Proc Natl Acad Sci USA 96:9727-9732.

Eyre D & Wu J-J (1987) Type XI or 1α2α3α collagen. In Burgeson RE & Mayne R (eds) Structure and Function of Collagen Types. Academic Press, Orlando, p 261-281.

Eyre D (2002) Collagen of articular cartilage. Arthritis Res 4:30-35.

Eyre DR (2004) Collagens and cartilage matrix homeostasis. Clin Orthop Rel Res 427 Suppl:S118-122.

Fairbank T (1947) Dysplasia epiphysialis multiplex. Proc R Soc Med (Ortho Sec) 39:315-317.

Felson DT & Zhang Y (1998) An update on the epidemiology of knee and hip osteoarthritis with a view to prevention. Arthritis Rheum 41:1343-1355.

Felson DT (2000) Osteoarthritis: new insights. Part 1: The disease and its risk factors. Ann Intern Med 133:635-646.

Felson DT (2004) Risk factors for osteoarthritis. Clin Orthop Rel Res 427 Suppl:S16-21

Felson DT, Couropmitree NN, Chaisson CE, Hannan MT, Zhang Y, McAlindon TE, LaValley M, Levy D & Myers RH (1998b) Evidence for a mendelian gene in a segregation analysis of generalized radiographic osteoarthritis. Arthritis Rheum 41:1064-1071 .

Fichard A, Kleman J-P & Ruggiero F (1994) Another look at collagen V and XI molecules. Matrix Biology 14:515-531.

Fiedler J, Stove J, Heber F & Brenner RE (2002) Clinical phenotype and molecular diagnosis of multiple epiphyseal dysplasia with relative hip sparing during childhood (EDM2) Am J Med Genet 112:144-53.

Forlino A, Piazza R, Tiveron C, Torre SD, Tatangelo L, Bonafé L, Gualeni B, Romano A, Pecora F, Superti-Furga A, Cetta A & Rossi A (2005) A diastrophic dysplasia sulfate transporter (SLC26A2) mutant mouse: morphological and biochemical characterization of the resulting chondrodysplasia phenotype. Hum Mol Genet, in press (Published on-line 9.2.2005).

Forster T, Chapman K & Loughlin J (2004a) Common variants within the interleukin 4 receptor α gene (IL4R) are associated with susceptibility to osteoarthritis. Hum Genet 114:391-395.

Forster T, Chapman K, Marcelline L, Mustafa Z, Southam L & Loughlin J (2004b) Finer linkage mapping of primary osteoarthritis susceptibility loci on chromosomes 4 and 16 in families with affected women. Arthritis Rheum 50:98-102.

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

Gaiser KG, Maddox BK, Bann JG, Boswell BA, Keene DR, Garofalo S & Horton WA (2002) Y-position collagen II mutation disrupts cartilage formation and skeletal development in a transgenic mouse model of spondyloepiphyseal dysplasia. J Bone Miner Res 17:39-47.

Ganguly A (2002) An update on conformation sensitive gel electrophoresis. Hum Mutat 19:334-342.

Ganguly A, Rock MJ & Prockop DJ (1993) Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. Proc Natl Acad Sci USA 90:10325-10329.

Garofalo S, Vuorio E, Metsaranta M, Rosati R, Toman D, Vaughan J, Lozano G, Mayne R, Ellard J, Horton W & de Crombrugghe B (1991) Reduced amounts of cartilage collagen fibrils and growth plate anomalies in transgenic mice harbouring a glycine-to-cysteine mutation in the mouse type II procollagen alpha-1-chain gene. Proc Natl Acad Sci 88:9648-9652.

Gelse K, Pöschl E & Aigner T (2003) Collagens – structure, function and biosynthesis. Adv Drug Deliv Rev 55:1531-1546.

Gillaspy E, Spreckley K, Wallis G, Doherty M & Spector TD (2002) Investigation of linkage on chromosome 2q and hand and knee osteoarthritis. Arthritis Rheum 46:3386-3389.

Goldring MB & Berenbaum F (2004) The regulation of chondrocyte function by proinflammatory mediators. Clin Orthop Rel Res 427 Suppl:S37-S46.

Goldring MB, Birkhead J, Sandell LJ, Kimura T & Krane SM (1988) Interleukin-1 suppresses expression of cartilage-specific types II and IX collagens and increases types I and III collagens in human chondrocytes. J Clin Invest 82:2026-2037.

Goldring MB, Fukuo K, Birkhead JR, Dudek E & Sandell LJ (1994) Transcriptional suppression by interleukin-1 and interferon-γ of type II collagen gene expression in human chondrocytes. J Cell Biochem 54:85-99.

Goldring MB (2000) The role of the chondrocyte in osteoarthritis. Arthritis Rheum 43:1916-1926.

Goldring S & Goldring MB (2004) The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clin Orthop Rel Res 427 Suppl:S27-236.

Gregory KE, Keene DR, Tufa SF & Lunstrum GP (2001) Developmental distribution of collagen type XII in cartilage: association with articular cartilage and the growth plate. J B Miner Res 16:2005-2016.

Gregory KE, Oxford JT, Chen Y, Gambee JE, Gygi SP, Aebersold R, Neame PJ, Mechling DE, Bächinger HP & Morris NP (2000) Structural organization of distinct domains within the non-collagenous N-terminal region of collagen type XI. J Biol Chem 275:11498-11506.

Griffith AJ, Sprunger LK, Sirko-Osadsa DA, Tiller GE, Meisler MH & Warman ML (1998) Marshall syndrome associated with a splicing defect at the *COL11A1* gene. Am J Hum Genet 62:816-823.

Guo Y, Bozic D, Malaskhkevich VN, Kammerer RA, Schulthess T & Engel J (1998) All-trans retinol, vitamin D and other hydrophobic compounds bind in the axial pore of the five-stranded coiled-coil domain of cartilage oligomeric matrix protein. EMBO J 17:5265-5272.

Haara MM, Manninen P, Kröger H,Arokoski JPA, Kärkkäinen A, Knekt P, Aromaa A & Heliövaara M (2003) Osteoarthritis of finger joints in Finns aged 30 or over: prevalence, determinants, and association with mortality. Ann Rheum Dis 62:151-158.

Hagg R, Hedbom E, Möllers U, Aszódi A, Fässler R & Bruckner P (1997) Absence of the α1(IX) chain leads to a functional knock-out of the entire collagen IX protein in mice. J Biol Chem 272:20650-20654.

Haila S, Hästbacka J, Böhling T, Karjalainen-Lindsberg ML, Kere J & Saarialho-Kere U (2001) SLC26A2 (diastrophic dysplasia sulfate transporter) is expressed in developing and mature cartilage but also in other tissues and cell types. J Histochem Cytochem 49:973-982.

Hanke M & Wink M (1994) Direct DNA sequencing of PCR-amplified vector inserts following enzymatic degradation of primers and dNTPs. Biotechniques 17:858-860.

Hanson IM, Gorm P, Lui VCH, Cheah KSE, Solomon E & Trowsdale J (1989) The human α2(XI) collagen gene (COL11A2) maps to the centromeric border of the major histocompatibility complex on chromosome 6. Genomics 5:925-931.

Harel T, Rabinowitz R, Hendler N, Galil A, Flusser H, Chemke J, Gradstein L, Lifshitz T, Ofir R, Elbedour K & Birk OS (2005) COL11A2 mutation associated with autosomal recessive Weissenbacher-Zweymüller syndrome: molecular and clinical overlap with otospondylomegaepiphyseal dysplasia (OSMED) Am J Med Genet 132:33-35.

Hartmann C & Tabin CJ (2000) Dual roles of Wnt signalling during chondrogenesis in the chicken limb. Development 127:3141-3159.

Hashimoto Y, Tomuyama T, Yamano Y & Mori H (2003) Mutation (D472Y) in the type 3 repeat domain of cartilage oligomeric matrix protein affects it early vesicle trafficking in endoplasmic reticulum and induces apoptosis. Am J Path 163:101-110..

Hauser N, Paulsson M, Heinegård D & Mörgelin M (1996) Interaction of cartilage matrix protein with aggrecan. Increased covalent cross-linking with tissue maturation. J Biol Chem 271:32247-32252.

Hecht JT, Hayes E, Haynes R & Cole WG (2005) COMP mutations, chondrocyte function and cartilage matrix. Matrix Biol 525-533.

Hecht JT, Makitie O, Hayes E, Haynes R, Susic M, Montufar-Solis D, Duke PJ & Cole WG (2004) Chondrocyte cell death and intracellular distribution of COMP and type IX collagen in the pseudoachondroplasia growth plate. J Orthop Res 22:759-767.

Hecht JT, Montufar-Solis D, Decker G, Lawler J, Daniels K & Duke PJ (1998) Retention of cartilage oligomeric matrix protein (COMP) and cell death in redifferentiated pseudoachondroplasia chondrocytes. Matrix Biol 17:625-633.

Hecht JT, Nelson LD, Crowder E, Wang Y, Elder FF, Harrison WR, Francomano CA, Prange CK, Lennon GG, Deere M & Lawler J (1995) Mutations in exon 17B of cartilage oligomeric matrix protein (COMP) cause pseudoachondroplasia. Nat Genet 10:325-429.

Hedbom E, Antonsson P, Hjerpe A, Aeschlimann D, Paulsson M, Rosa-Pimentel E, Sommarin Y, Wendel Y, Oldberg Å & Heinegård D (1992) Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. J Biol Chem 267:6232-6136.

Heinegård D & Oldberg Å (1989) Structure and biology of cartilage and bone matrix noncollagenous macromolecules. FASEB J 3:2042-2051.

Heliövaara M (1996) Raajanivelten nivelrikko. In: Tuki- ja liikuntaelinsairadet Suomessa. Ongelman laajuus, syyt sekä ehkäisy-, hoito- ja kuntoutumismahdollisuudet. Sosiaali- ja terveysministeriön työryhmämuistioita, Helsinki, 18:19-23.

Helminen HJ, Kiraly K, Pelttari A, Tammi MI, Vandenberg P, Pereira P, Dhulipala R, Khillan JS, Ala-Kokko L, Hume EL, Sokolov BP & Prockop DJ (1993) An inbred line of transgenic mice expressing an internally deleted gene for type II procollagen (COL2A1): young mice have a variable phenotype of a chondrodysplasia and older mice have osteoarthritic changes in joints. J Clin Invest 92:582-595.

Helminen HJ, Säämänen A-M, Salminen H & Hyttinen MM (2002) Transgenic mouse models for studying the role of cartilage macromolecules in osteoarthritis. Rheumatology 41:848-856.

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

Hirsch R, Lethbridge-Cejku M, Hanson R, Scott WW Jr., Reichle R, Plato CC, Tobin JD & Hochberg MC (1998) Familial aggregation of osteoarthritis. Arthritis Rheum 41:1227-1232.

Holden P, Canty EG, Mortier GR, Zabel B, Spranger J, Carr A, Grant ME, Loughlin J & Briggs MD (1999) Identification of novel pro-alpha2 (IX) collagen gene mutations in two families with distinctive oligoepiphyseal forms of multiple epiphyseal dysplasia. Am J Hum Genet 65:31-38.

Holden P, Meadows RS, Chapman KL, Grant ME, Kadler KE & Briggs MD (2001) Cartilage oligomeric matrix protein interacts with type IX collagen, and disruptions to these interactions identify a pathogenetic mechanism in a bone dysplasia family. J Biol Chem 276:6046-6055.

Holderbaum D, Bleasel JF, Jonsson H, Saxne T, Prockop DJ, Williams CJ & Moskowitz RW (1996) Arg519-Cys-associated haplotype in Icelandic family suggests early origin of this mutation. Matrix Biol 1996:15:178.

Hollander AP, Heathfield TF, Webber C, Iwata Y, Bourne R, Rorabeck C & Poole AR (1994) Increased damage to type II collagen in osteoarthritis articular cartilage detected by a new immunoassay. J Clin Invest 93:1722-1732.

Hollander AP, Pidoux I, Reiner A, Rorabeck C, Bourne R & Poole AR (1995) Damage to type II collagen in ageing and osteoarthritis starts at the articular surface, originates around chondrocytes and extends into the cartilage with progressive degeneration. J Clin Invest 96:2859-2869.

Horton WA (2003) Skeletal development: insights from targeting the mouse genome. Lancet 362:560-569.

Hou J, Putkey JA & Hecht JT (2000) Delta 469 mutation in the type 3 repeat calcium binding domain of cartilage oligomeric matrix protein (COMP) disrupts calcium binding. Cell Calcium 27:309-314.

Huber C, Odent S, Rumeur S, Padovani P, Penet C, Cormier-Daire V, Munnich A & Le Merrer M (2001) Sulphate transporter gene mutations in apparently isolated club foot. J Med Genet 38:191-192.

Huber M, Trattnig S & Lintner F (2000) Anatomy, biochemistry, and physiology of articular cartilage. Invest Radiol 35:573-580.

Huber S, van der Rest M, Bruckner P, Rodriquez E, Winterhalter KH & Vaughan L (1986) Identification of type IX collagen polypeptide chains. The alpha2(IX) polypeptide carries the chondroitin sulfate chain(s) J Biol Chem 261:5965-5968.

Hunter DJ, Demissie S, Cupples LA, Aliabadi P & Felson DT (2004) A genome scan for joint-spesific hand osteoarthritis susceptibility. Arthritis Rheum 50:2489-2496.

Hunter DJ, Snieder H, March L & Sambrook PN (2003) Genetic contribution to cartilage volume in women: a classical twin study. Rheumatology 42:1495-1500.

Hästbacka J, de la Chapelle A, Mahtani MM, Clines G, Reeve-Daly MP, Daly M, Hamilton BA, Kusumi K, Trivedi B, Weaver A, Coloma A, Lovett M, Buckler A, Kaitila I & Lander ES (1994) The diastrophic dysplasia gene encodes a nove sulphate transporter: positional cloning by fine-structure linkage disequilibrium mapping. Cell 78: 1073-1087.

Hästbacka J, Kerrebrock A, Mokkala K, Clines G, Lovett M, Kaitila I, de la Chapelle A & Lander ES (1999) Identification of the Finnish founder mutation for diastrophic dysplasia (DTD) Eur J Hum Genet 7:664-670.

Höglund P, Haila S, Socha J, Tomaszewski L, Saariaho-Kere U, Karjalainen-Lindsberg M-L, Airola K, Holmberg C, de la Chapelle A & Kere J (1996) Mutations of the down-regulated in adenoma (DRA) gene cause congenital chloride diarrhea. Nature Genet 14:316-319.

Igekawa S (2004) Another family with multiple epiphyseal dysplasia harbouring the R718W mutation in COMP. J Med Genet E-letter 25.6.2004.

Igekawa S, Ohashi H, Nishimura G, Kim KC, Sannohe A, Kimizuka M, Fukushima Y, Nagai T & Nakamura Y (1998) Novel and recurrent COMP (cartilage oligomeric matrix protein) mutations in pseudoachondroplasia and multiple epiphyseal dysplasia. Hum Genet 103:633-638.

Ikeda T, Mabuchi A, Fukuda A, Kawakami A, Yamada R, Yamamoto S, Miyoshi K, Haga N, Hiraoka H, Takatori Y, Kawakuchi H, Nakamura K & Igekawa S (2002) Association analysis of single nucleotide polymorphisms in cartilage-specific collagen genes with knee and hip osteoarthritis in the Japanese population. J Bone Miner Res 17:1290-1296.

Imai K, Ohta S, Matsumoto T, Fujimoto N, Sato H, Seiki M & Okada Y (1997) Expression of membrane-type I matrix metalloproteinase and activation of progelatinase A in human osteoarthritic cartilage. Am J Pathol 151:245-256.

Ingvarsson T (2000) Prevalence and inheritance of hip osteoarthritis in Iceland. Acta Orthop Scand Suppl 298:1-46.

Ingvarsson T, Stefánsson SE, Gulcher JR, Jónsson HH, Jónsson H, Frigge ML, Pálsdóttir E, Ólafsdóttir G, Jónsdóttir P, Walters GB, Lohmander LS & Stefánsson K (2001) A large Icelandic family with early osteoarthritis of the hip associated with a susceptibility locus on chromosome 16p. Arthritis Rheum 44:2548-2555.

Ingvarsson T, Stefansson SE, Hallgrimsdottir IB, Frigge ML, Johnsson H Jr., Gulcher J, Jonsson H, Ragnarsson JI, Lohmander LS & Stefansson K (2000) The inheritance of hip osteoarthritis in Iceland. Arthritis Rheum 43:2785-2792.

Ito MM & Kida MY (2000) Morphological and biochemical re-evaluation of the process of cavitation in the rat knee joint: cellular and cell strata alterations in the interzone. J Anat 194 Part 4:659-679.

Jackson GC, Barker FS, Jakkula E, Czarny-Ratajczak M, Mäkitie O, Cole WG, Wright MJ, Smithson SF, Suri M, Rogala P, Mortier GR, Baldock C, Wallace A, Elles R, Ala-Kokko L & Briggs MD (2004) Missense mutations in the β-strands of the single A-domain of matrilin-3 result in multiple epiphyseal dysplasia. J Med Genet 41:52-59.

Kalichman L, Kobyliansky E, Malin I, Yakovenko K& Livshits G (2003) Search for linkage between hand osteoarthritis and 11q12-13 chromosomal segment. Osteoarthritis Cartilage 11:561-568.

Kavanagh E, Abiri M, Bland YS & Ashhurst DE (2002) Division and death of cells in developing synovial joints and long bones. Cell Biol Int 26:679-688.

Kawaji H, Nishimura G, Watanabe S, Mabuchi A, Ikeda T, Ohashi H, Sasaki A, Sano T & Igekawa S (2002) Autosomal dominant precocious osteoarthropathy due to a mutation of the cartilage oligomeric matrix protein (COMP) gene: further expansion of the phenotypic variations of COMP defects. Skeletal Radiol 31:730-737.

Keen RW, Hart DJ, Lanchbury JS & Spector TD (1997) Association of early osteoarthritis of the knee with a *Taq* I polymorphism of the vitamin D receptor gene. Arthritis Rheum 40:1444-1449.

Keene DR, Engvall E & Glanville RW (1988) Ultrastructure of type VI collagen in human skin and cartilage suggests an anchoring function for this filamentous network. J Cell Biol 107:1995-2005.

Keene DR, Oxford JT & Morris NP (1995) 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 43:967-979.

Kellgren JH, Lawrence JS & Bier F (1963) Genetic factors in generalised osteoarthritis. Ann Rheum Dis 22:237-255.

Kimura T, Mattei MG, Stevens JW, Goldring MB, Ninomiya Y & Olsen BR (1989) Molecular cloning of rat and human type IX collagen cDNA and localization of the alpha 1(IX) gene on the human chromosome 6. Eur J Biochem 179:71-78.

Kimura T, Nakata T, Tsumaki N, Miyamoto S, Matsui Y, Ebara S & Ochi T (1996) Progressive degeneration of articular cartilage and intervertebral discs. An experimental study in transgenic mice bearing a type IX collagen mutation. Int Orthop 20:177-181.

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

Kizawa H, Kou I, Iida A, Sudo A, Miyamoto Y, Fukuda A, Mabuchi A, Kotani A, Kawakami A, Yamamoto S, Uchida A, Nakamura K, Notoya K, Nakamura Y & Ikegawa S (2005) An aspartic acid repeat polymorphism in asporin inhibits chondrogenesis and increases susceptibility to osteoarthritis. Nature Gen, in press (published on-line 9.1.2005).

Klatt AR, Nitsche DP, Kobbe B, Mörgelin M, Paulsson M &Wagener R (2000) Molecular structure and tissue distribution of matrilin-3, a filament-forming extracellular matrix protein expressed during skeletal development. J Biol Chem 275:3999-4006.

Klatt AR, Paulsson M & Wagener R (2002) Expression of matrilins during maturation of mouse skeletal tissues. Matrix Biology 21:289-296.

Kleemann-Fischer D, Kleemann GR, Engel D, Yates III JR, Wu J-J & Eyre DR (2001) Molecular properties of matrilin-3 isolated from human growth cartilage. Arch Biochem Biophys 387:209-215.

Kleerekoper Q, Hecht JT & Putkey JA (2002) Disease-causing mutations in cartilage oligomeric matrix protein cause an unstructured Ca^{2+} binding domain. J Biol Chem 277:10581-10589.

Kleman J-P, Hartmann DJ, Ramirez F& van der Rest M (1992) The rhabdomyosarcoma cell line A204 lays down a highly insoluble matrix composed mainly of α1 type XI and α2 type V collagen chains. Eur J Biochem 210:329-335.

Knauper V, Lopez-Otin C, Smith B, Knight G & Murphy G (1996) Biochemical characterization of human collagenase-3. J Biol Chem 271:1544-1550.

Knudson CB & Knudson W (2001) Cartilage proteoglycans. Semin Cell Dev Biol 12:69-78.

Ko Y, Kobbe B, Nicolae C, Miosge N, Paulsson M, Wagener R & Aszódi A (2004) Matrilin-3 is dispensable for mouse skeletal growth and development. Mol Cell Biol 24:1691-1699.

Kornak U & Mundlos S (2003) Genetic disorders of the skeleton: a developmental approach. Am J Hum Genet 73:447-474.

Kronenberg H (2003) Developmental regulation of the growth plate. Nature 423:332-336.

Kuivaniemi H, Tromp G & Prockop DJ (1997) Mutations in fibrillar collagens (types I, II, III and XI), fibril-associated collagen (type IX) and network-forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels. Hum Mutat 9:300-315.

Kvansakul M, Adams JC & Hohenester E (2004) Structure of a thrombospondin C-terminal fragment reveals a novel calcium core in the type 3 repeats. EMBO J 23:1223-1233.

Körkkö J, Annunen S, Pihlajamaa T, Prockop DJ & Ala-Kokko L (1998) Conformation sensitive gel electrophoresis for simple and accurate detection of mutations: comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. Proc Natl Acad Sci USA 95:1681-1685.

Lachman RS, Krakow D, Cohn DH & Rimoin DL (2004) MED, COMP, multilayered and NEIN: an overview of multiple epiphyseal dysplasia. Pediatr Radiol 35:116-123.

Lai CH & Chu ML (1996) Tissue distribution and developmental expression of type XVI collagen in the mouse. Tissue Cell 28:155-164.

Lamb KJ, Lewthwaite JC, Bastow ER & Pitsillides AA (2003) Defining boundaries during joint cavity formation: going out on a limb. Int J Exp Pathol 84:55-67.

Lanyon P, Muir K, Doherty S & Doherty M (2000) Assessment of a genetic contribution to osteoarthritis of the hip: sibling study. BMJ 321:1179-1183.

Lark MW, Byner EU, Flanagan J, Harper CF, Hoerrner LA, Hutchinson NI, Singer II, Donatelli SA, Weidner HR, Williams HR, Mumford RA & Lohmander LS (1997) Aggrecan degradation in human cartilage. Evidence for both matrix metalloproteinase and aggrecanase activity in normal, osteoarthritic and rheumatoid joints. J Clin Invest 100: 93-106.

Law ML, Chan SDH, Berger R, Jones C, Kao FT, Solomon E & Cheah KSE (1990) The gene for the α2(XI) chain of collagen type XI (COL11A2) assigned to the short arm of chromosome 6. Am Hum Genet 54:23-29.

Lefebvre V, Peeters-Joris C & Vaes C (1990) Modulation by interleukin 1 and tumor necrosis factor α of production of collagenase, tissue inhibitor of metalloproteinases and collagen types in differentiated and dedifferentiated articular chondrocytes. Biochem Biophys acta 1052:366-378.

Leppävuori J, Kujala U, Kinnunen J, Kaprio J, Nissilä M, Heliövaara M, Klinger N, Partanen J, Terwilliger JD & Peltonen L (1999) Genome scan for predisposing loci for distal interphalangeal joint osteoarthritis: evidence for a locus on 2q. Am J Hum Genet 65:1060-1067.

Li S-W, Prockop DJ, Helminen HJ, Fassler R, Lapvetelainen T, Kiraly K, Peltarri A, Arokoski J, Lui H, Arita M & Khillan JS (1995) Transgenic mice with targeted inactivation of the *Col2a1* gene for collagen II develop a skeleton with membranous and periosteal bone but no endochondral bone. Genes Dev 9:2821-2830.

Li S-W, Takanosu M, Arita MM, Bao Y, Ren Z-X, Maier A, Prockop DJ & Mayne R (2001) Targeted disruption of *Col11a2* produces a mild cartilage phenotype in transgenic mice: comparison with the human disorder otospondylomegaepiphyseal dysplasi (OSMED) Dev Dyn 222:141-152.

Li Y & Olsen BR (1997) Murine models of human genetic skeletal disorders. Matrix Biol 16:49-52.

Lindberg H (1986) Prevalence of primary coxarthrosis in siblings of patients with primary coxarthrosis. Clin Orthop 203:273-275.

Lohi H, Kujala M, Kerkelä E, Saarialho-Kere U, Kestilä M & Kere J (2000) Mapping of five new putative anion transporter genes in human and characterization of SLC26A6, a candidate gene for pancreatic anion exchanger. Genomics 70:102-112.

Lohiniva J, Paassilta P, Seppanen U, Vierimaa O, Kivirikko S & Ala-Kokko L (2000) Splicing mutations in the COL3 domain of collagen IX cause multiple epiphyseal dysplasia. Am J Med Genet 90:216-222.

Loughlin J (2001) Genetic epidemiology of primary osteoarthritis. Curr Opin Rheumatol 13:111-116.

Loughlin J (2002a) Genome studies and linkage in primary osteoarthritis. Rheum Dis Clin North Am 28:95-109.

Loughlin J (2003) Genetics of osteoarthritis and potential for drug development. Curr Opin Pharmacol 3:295-299.

Loughlin J, Dowling B, Chapman K, Marcelline L, Mustafa Z, Southam L, Ferreira A, Ciesielski C, Carson DA & Corr M (2004) Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. Proc Natl Acad Sci USA 101:9757-9762.

Loughlin J, Dowling B, Mustafa Z & Chapman K (2002b) Association of the interleukin-1 gene cluster on chromosome 2q13 with knee osteoarthritis. Arthritis Rheum 46:1519-1527.

Loughlin J, Dowling B, Mustafa Z, Southam L & Chapman K (2002d) Refined linkage mapping f a hip osteoarthritis susceptibility locus on chromosome 2q. Rheumatology 41:955-956.

Loughlin J, Irven C, Mustafa Z, Briggs MD, Carr A, Lynch SA, Knowlton RG, Cohn DH & Sykes B (1998) Identification of five novel mutations in cartilage oligomeric matrix protein gene in pseudoachondroplasia and multiple epiphyseal dysplasia. Hum Mutat Suppl1:S10-S17.

Loughlin J, Mustafa Z, Dowling B, Southam L, Marcelline L, Räinä SS, Ala-Kokko L & Chapman K (2002c) Finer linkage mapping of a primary hip osteoarthritis susceptibility locus on chromosome 6. Eur J Hum Genet 10:562-568.

Loughlin J, Mustafa Z, Irven C, Smith A, Carr AJ, Sykes B & Chapman K (1999) Stratification analysis of an osteoarthritis genome screen – suggestive linkage to chromosomes 4,6 and 16. Am J Hum Genet 65:1795-1798.

Loughlin J, Mustafa Z, Smith A, Irven C, Carr AJ, Clipsham K, Chitnavis J, Bloomfield VA, McCartney M, Cox O, Sinsheimer JS, Sykes B & Chapman K (2000) Linkage analysis of chromosome 2q in osteoarthritis. Rheumatology 39:377-381.

Lui VCH, Kong RYC, Nicholls J, Cheung ANY & Cheak KSE (1995) The mRNAs for the three chains of human collagen type XI are widely distributed but not necessarily co-expressed: implications for homotrimeric, heterotrimeric and heterotypic collagen molecules. Biochem J 311:511-516.

Lui VCH, Ng LJ, Sat EWY, Nicholls J & Cheah KSE (1996) Extensive alternative splicing within the amino-propeptide coding domain of α2(XI) procollagen mRNAs. Expression of transcripts encoding truncated pro-α chains. J Biol Chem 271:16945-16951.

Löppönen T, Körkkö J, Lundan T, Seppänen U, Ignatius J & Kääriäinen H (2004) Childhood-onset osteoarthritis, tall stature and sensorineural hearing loss associated with Arg^{75}-Cys mutation in procollagen type II gene (COL2A1) Arthritis Rheum 51:925-932.

Mabuchi A, Haga N, Maeda K, Nakashima E, Manabe N, Hiraoka H, Kitoh H, Kosaki R, Nishimura G, Ohashi H & Igekawa S (2004) Novel and recurrent mutations clustered in the von Willebrand factor A domain of MATN3 in multiple epiphyseal dysplasia. Hum Mutat Mutation in brief #757 online.

Mabuchi A, Manabe N, Haga N, Kitoh H, Ikeda T, Kawaji H, Tamai K, Hamada J, Nakamura S, Brunetti-Pierri N, Kimizuka M, Takatori Y, Nakamura K, Nishimura G, Ohashi H & Igekawa S (2003) Novel types of COMP mutations and genotype-phenotype association in pseudoachondroplasia and multiple epiphyseal dysplasia. Hum Genet 112:84-90.

MacGregor AJ & Spector TD (1999) Twins and the genetic architecture of osteoarthritis. Rheumatology 38:583-590.

MacGregor AJ, Antoniades L, Matson M, Andrew T & Spector TD (2000) The genetic contribution to radiographic hip osteoarthritis in women: results of a classic twin study. Arthritis Rheum 43:2410-2416.

Maddox BK, Keene DR, Sakai LY, Charbonneau NL, Morris NP, Ridgway CC, Boswell BA, Sussman MD, Horton WA, Bächinger HP & Hecht JT (1997) The fate of cartilage oligomeric matrix protein is determined by the cell type in the case of a novel mutation in pseudoachondroplasia. J Biol Chem 272:30993-30997.

Maddox BK, Mokashi A, Keene DR & Bächinger HP (2000) A cartilage oligomeric matrix protein mutation associated with pseudoachondroplasia changes the structural and functional properties of the type 3 domain. J Biol Chem 275:11412-11417.

Maeda S, Ishidou Y, Koga H, Taketomi E, Ikari K, Komiya S, Takeda J, Sakou T & Inoue I (2001a) Functional impact of human collagen α2(XI) gene polymorphism in pathogenesis of ossification of the posterior longitudinal ligament of the spine. J Bone Miner Res 16:948-957.

Maeda S, Koga H, Matsunaga S, Numasawa T, Ikari K, Furushima K, Harata S, Takeda J, Sakou T, Komiya S & Inoue I (2001b) Gender-spesific haplotype association of collagen α2(XI) gene in ossification of the posterior longitudinal ligament of the spine. J Hum Genet 46:1-4.

Malaschkevich VN, Kammerer RA, Efimov VP, Schulthess T & Engel J (1996) The crystal structure of a five-stranded coiled coil in COMP: a prototype ion channel? Science 274:761-765.

Mann HH, Özbek S, Engel J, Paulsson M & Wagener R (2004) Interactions between the cartilage oligomeric matrix protein and matrilins. Implications for matrix assembly and the pathogenesis of chondrodysplasias. J Biol Chem 279:25294-25298.

Martel-Pelletier J (2004) Pathophysiology of osteoarthritis. Osteoarthritis Cartilage 12 Suppl:S31-S33.

Mayne R, Brewton RG, Mayne PM & Baker JR (1993) Isolation and characterization of the chains of type V/type XI collagen present in bovine vitreous. J Biol Chem 268:9381-9386.

McCormick D, van der Rest M, Goodship J, Lozano G, Ninomiya Y & Olsen BR (1987) Structure of the glycosaminoglycan domain in the type IX collagen-proteoglycan. Proc Natl Acad Sci USA 84:4044-4048.

McGuirt WT, Prasad SD, Griffith AJ, Kunst HPM, Green GE, Shpargel KB, Runge C, Huybrechts C, Mueller RF, Lynch E, King M-C, Brunner HG, Cremers CWRJ, Takanosu M, Li S-W, Arita M, Mayne R, Prockop DJ, Van Camp G & Smith RJH (1999) Mutations in *COL11A2* cause non-syndromic hearing loss (DFNA13) Nature Gen 23:413-419.

McLean W & Olsen BR (2001) Mouse models of abnormal skeletal development and homeostasis. Trends in Genet 17:S38-S43.

Medeck RJ, Sosa S, Morris NP & Oxford JT (2003) BMP-1-mediated proteolytic processing of alternatively spliced isoforms of collagen type XI. Biochem J 376:361-368.

Melkoniemi M, Brunner HG, Manovrier S, Hennekam R, Superti-Furga A, Kääriäinen H, Pauli RM, van Essen T, Warman ML, Bonaventure J, Miny P & Ala-Kokko L (2000) Autosomal recessive disorder otospondylomegaepiphyseal dysplasia is associated with loss-of-function mutations in the COL11A2 gene. Am J Hum Genet 66:368-377.

Melkoniemi M, Koillinen H, Männikkö M, Warman ML, Pihlajamaa T, Kääriäinen H, Rautio J, Hukki J, Stofko JA, Cisneros GJ, Krakow D, Cohn DH, Kere J & Ala-Kokko L (2003) Collagen XI sequence variations in nonsyndromic cleft palate, Robin sequence and micrognathia. Eur J Hum Genet 11:265-270.

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

Metsäranta M, Garofalo S, Decker G, Rintala M, DeCrombrugghe B &Vuorio E (1992) Chondrodysplasia in mice harbouring a 15 amino acid deletion in triple helical domain of proα1(II) collagen chain. J Cell Biol 118:203-212.

Meulenbelt I, Seymour AB, Nieuwland M, Huizinga TWJ, van Duijn CM & Slagboom PE (2004) Association of the interleukin-1 gene cluster with radiographic signs of osteoarthritis of the hip. Arthritis Rheum 50:1179-1186.

Meyer FA, Yaron I & Yaron M (1990) Synergistic, additive, and antagonistic effects of interleukin-1β, tumor necrosis factor α, and γ-interferon on prostaglandin E, hyaluronic acid, and collagenase production by cultured synovial fibroblasts. Arthritis Rheum 33:1518-1525.

Mier RJ, Holderbaum D, Ferguson R & Moskowitz R (2001) Osteoarthritis in children associated with a mutation in the type II procollagen gene (COL2A1). Mol Genet Metab 74:338-41.

Mikkelsen WM & Duff IF (1970) Age-sex prevalence of radiographic abnormalitis of the joints of the hand, wrist and cervical spine of adult resident of Tecumseh. Michigan community Health sudy area 1962-1965. J Chronic Dis 23:151-159.

Mitchell PG, Magna HA, Reeves LM, Lopresti-Morrow LL, Yocum SA, Rosner PJ, Geoghan KF & Hambor JE (1996) Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. J Clin Invest 97:761-768.

Mitrovic D (1977) Development of the metatarsophalangeal joint of the chick embryo: morphological, ultrastructural and histochemical studies. Am J Anat 150:333-347.

Mitrovic D (1978) Development of the diarthrodial joints in the rat embryo. Am J Anat 151:475-485.

Mizoguchi I, Nakamura M, Takahashi I, Kagayama M & Mitani H (1990) An immunohistochemical study of localization of type I and II collagens in mandibular condylar cartilage compared with tibial growth plate. Histochemistry 93:593-599.

Morris NP & Bächinger HP (1987) Type XI collagen is a heterotrimer with the composition (1α2α3α) retaining non-triple-helical domains. J Biol Chem 262:11345-11350.

Morris NP, Oxford JT, Davies GBM, Smoody BF & Keene DG (2000) Developmentally regulated alternative splicing of the α1(XI) collagen chain: spatial and temporal segregation of isoforms in the cartilage of fetal rat long bones. J Histochem Cytochem 48:725-741.

Mortier GR, Chapman K, Leroy JL & Briggs MD (2001) Clinical and radiographic features of multiple epiphyseal dysplasia not linked to the COMP or type IX collagen genes. Eur J Hum Genet 9:606-12.

Moskowitz RW (2004) Spesific gene defects leading to osteoarthritis. J Rheumatol 31:supplement 70:16-21.

Mostert AK, Dijkstra PF, Jansen BR, van Horn JR, de Graaf B, Heutink P & Lindhout D (2003) Familial multiple epiphyseal dysplasia due to a matrilin-3 mutation: further delineation of the phenotype including 40 years follow-up. Am J Med Genet 120A:490-497.

Muller G, Michel A & Altenburg E (1998) COMP (cartilage oligomeric matrix protein) is synthesized in ligament, tendon, meniscus and articular cartilage. Conn Tiss Res 39:233-244.

Mundlos S, Meyer R, Yamada Y & Zabel B (1991) Distribution of cartilage proteoglycan (aggrecan) core protein and link protein gene expression during human skeletal development. Matrix 11:339-346.

Muragaki Y, Mariman EC, van Beersum SE, Perälä M, van Mourik JB, Warman ML, Olsen BR & Hamel BC (1996) A mutation in the gene encoding the alpha 2 chain of the fibril-associated collagen IX, COL9A2, causes multiple epiphyseal dysplasia (EDM2) Nat Genet 12:103-105.

Murphy G, Knäuper V, Atkinson S, Butler G, English W, Hutton M, Stracke J & Clark I (2002) Matrix metalloproteinases in arthritic disease. Arthritis Res 4 (suppl 3):S39-S49.

Murray PD & Drachman DB (1969) The role of movement in the development of joints and related structures: the head and neck in the chick embryo. J Embryol Exp Morph 22:349-371.

Mustafa Z, Chapman K, Irven C, Carr AJ, Clipsham K, Chitnavis J, Sinsheimer JS, Bloomfield VA, McCartney M, Cox O, Sykes B & Loughlin J (2000) Linkage analysis of candidate genes as susceptibility loci for osteoarthritis – suggestive linkage of COL9A1 to female hip osteoarthritis. Rheumatology 39:299-306.

Myllyharju J & Kivirikko KI (2001) Collagens and collagen-related diseases. Ann Med 33:7-21.

Myllyharju J & Kivirikko KI (2004) Collagens, modifying enzymes and their mutations in humans, flies and worms. Trends Genet 20:33-43.

Mäkitie O, Mortier GR, Czarny-Ratajczak M, Suri M, Rogala P, Jakkula E, Ala-Kokko L, Briggs MD & Cole WG (2004) Clinical and radiographic findings in multiple epiphyseal dysplasia caused by MATN3 mutations: description of 12 patients. Am J Med Genet 125A:278-284.

Mäkitie O, Savarirayan R, Bonafe L, Robertson S, Susic M, Superti-Furga A & Cole WG (2003) Autosomal recessive multiple epiphyseal dysplasia with homozygosity for C653S in the DTDST gene: double-layer patella as a reliable sign. Am J Med Genet 122A:187-192.

Mörgelin M, Heinegård D, Engel J & Paulsson M (1992) Electron microscopy of native cartilage oligomeric matrix protein purified from the swarm rat chondrosarcoma reveals a five-armed structure. J Biol Chem 267:6137-6141.

Nagase H & Kashiwagi M (2003) Aggrecanases and cartilage matrix degradation. Arthritis Res Ther 5:94-103.

Nah HD, Berenbaum M & Upholt WB (1992) The chicken α1(XI) collagen gene is widely expressed in embryonic tissues. J Biol Chem 267:22581-22586.

Nakashima E, Ikegawa S, Ohashi H, Kimizuka M & Nishimura G (2005) Double-layered patella in multiple epiphyseal dysplasia is not exclusive to DTDST mutation. Am J Med Genet 133:106-107.

Nakashima E, Kitoh H, Maeda K, Haga N, Kosaki R, Mabuchi A, Nishimura G, Ohashi H & Igekawa S (2005) Novel COL9A3 mutation in a family with multiple epiphyseal dysplasia. Am J Med Genet 132A:181-184.

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

Nalin AM, Greenlee TK Jr & Sandell LJ (1995) Collagen gene expression during development of avian synovial joints: transient expression of types II and XI collagen genes in the joint capsule. Dev Dyn 203:352-262.

Neame RL, Muir K, Doherty S & Doherty M (2004) Genetic risk of knee osteoarthritis: a sibling study. Ann Rheum Dis 63:1022-1027.

Newman B & Wallis GA (2002) Is osteoarthritis a genetic disease? Clin Invest Med 25:139-49.

Newton G, Weremowicz S, Morton CC, Copeland NG, Gilbert DJ, Jenkins NA & Lawler J (1994) Characterization of human and mouse cartilage oligomeric matrix protein. Genomics 24:435-439.

Noponen-Hietala N, Kyllönen E, Männikkö M, Ilkko E, Karppinen J, Ott J & Ala-Kokko L (2003) Sequence variations in the collagen IX and XI genes are associated with degenerative lumbar spinal stenosis. Ann Rheum Dis 62:1208-1214.

Oldberg Å, Antonsson P, Lindblom K & Heinegård D (1992) COMP (cartilage oligomeric matrix protein) is structurally related to the thrombospondins. J Biol Chem 267:22346-22350.

Olsen BR, Reginato A & Wang W (2000) Bone development. Annu Rev Cell Dev Biol 16:191-220.

Oxford JT, Doege KJ & Morris NP (1995) Alternative exon splicing within the amino-terminal non-triple-helical domain of the rat pro-α(XI) collagen chain generates multiple forms of the mRNA transcript which exhibit tissue-dependent variation. J Biol Chem 270:9478-9485.

Paassilta P, Lohiniva J, Annunen S, Bonaventure J, Le Merrer M, Pai L & Ala-Kokko L (1999b) COL9A3: a third locus for multiple epiphyseal dysplasia. Am J Hum Genet 64:1036-1044.

Paassilta P, Lohiniva J, Göring HHH, Perälä M, Räinä SS, Karppinen J, Hakala M, Palm T, Kröger H, Kaitila I, Vanharanta H, Ott J & Ala-Kokko L (2001) Identification of a novel common genetic risk factor for lumbar disk disease. JAMA 285:1843-1849.

Paassilta P, Pihlajamaa T, Annunen S, Brewton RG, Wood BM, Johnson CC, Liu J, Gong Y, Warman ML, Prockop DJ, Mayne R & Ala-Kokko L (1999a) Complete sequence of the 23-kilobase human COL9A3 gene. Detection of Gly-X-Y triplet deletions that represent neutral variants. J Biol Chem 274:22469-22475.

Pace JM, Li Y, Seegmiller RE, Teuscher C, Taylor BA & Olsen BR (1997), Disproportionate micromelia (Dmm) in mice caused by a mutation in the C-propeptide coding region of Col2a1. Dev Dyn 208:25-33.

Peltonen L & McKusick VA (2001) Dissecting human disease in the postgenomic era. Science 291:1224-1228.

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

Pihlajamaa T, Lankinen H, Ylöstalo J, Valmu L, Jäälinoja J, Zaucke F, Spitznagel L, Gösling S, Puustinen A, Mörgelin M, Peränen J, Maurer P, Ala-Kokko L & Kilpeläinen I (2004) Characterization of recombinant amino-terminal NC4 domain of human collagen IX. J Biol Chem 279:24265-24273.

Pihlajamaa T, Prockop DJ, Faber J, Winterpacht A, Zabel B, Giedion A, Wiesbauer P, Spranger J & Ala-Kokko L (1998b) Heterozyous glycine substitution in the COL11A2 gene in the original patient with the Weissenbacher-Zweymüller syndrome demonstrates its identity with heterozygous OSMED (nonocular Stickler syndrome) Am J Med Genet 80:115-120.

Pihlajamaa T, Vuoristo MM, Annunen S, Perälä M, Prockop DJ & Ala-Kokko L (1998a) Two genes of 90 and 15 kb code for similar polypeptides of the same collagen molecule. Matrix Biol 17:237-241.

Poole AR (1999) An introduction to the pathophysiology of osteoarthritis. Front Biosc 4:d662-670.

Poole AR, Kobayashi M, Yasuda T, Laverty S, Mwale F, Kojima T, Sakai T, Wahl C, El-Maadawy S, Webb G, Tchetina E & Wu W (2002) Type II collagen degeneration and its regulation in articular cartilage in osteoarthritis. Ann Rheum Dis 61(Suppl II):ii78-ii81.

Poole AR, Kojima T, Yasuda T, Mwale F, Kobayashi M & Laverty S (2001) Composition and structure of articular cartilage. Clin Orthop Rel Res 391 Suppl: S26-S33.

Poole AR, Rosenberg LC, Reiner A, Ionescu M, Bogoch E & Roughley PJ (1996) Contents and distributions of the proteoglycans decorin and biglycan in normal and osteoarthritic human articular cartilage. J Orthop Res 41:681-689.

Pullig O, Weseloh G, Klatt AR, Wagener R & Swoboda B (2002) Matrilin-3 in human articular cartilage: increased expression in osteoarthritis. Osteoarthritis Cartilage 10:253-63.

Pun YL, Moskowitz RW, Lie S, Sundstrom WR, Block SR, McEwen C, Williams HJ, Bleasel JF, Holderbaum D & Haqqi TM (1994) Clinical correlations of osteoarthritis associated with a single-base mutation (arginine^{519} to cysteine) in type II procollagen gene. Arthritis Rheum 37:264-269.

Ramensky V, Bork P & Sunyaev S (2002) Human non-synonymous SNPs: server and survey. Nucleic Acids Res 30:3894-900.

Reginato AM & Olsen BR (2002) The role of structural genes in the pathogenesis of osteoarthritic disorders. Arthritis Res 4:337-345.

Richards AJ, Yates JRW, Williams R, Payne SJ, Pope FM, Scott JD & Snead M (1996) A family with Stickler syndrome type 2 has a mutation in the *COL11A1* gene resulting in the substitution of glycine 97 by valine in alpha-1(XI) collagen. Hum Mol Genet 5:1339-1343.

Risch NJ (2000) Searching for genetic determinants in the new millennium. Nature 405:847-856.

Roach HI, Baker JE & Clarke NMP (1998) Initiation of the bony epiphysis in long bones: chronology of interactions between the vascular system and the chondrocytes. J Bone Min Res 13:950-961.

Roby P, Eyre E, Worthington J, Ramesar R, Cilliers H, Beighton P, Grant M & Wallis G (1999) Autosomal dominant (Beukes) premature degenerative osteoarthropathy of the hip joint maps to an 11 cM region on chromosome 4q35. Am J Hum Genet 64:904-908.

Rodriquez RR, Seegmiller RE, Stark MR & Bridgewater LC (2004) A type XI collagen mutation leads to increased degradation of type II collagen in articular cartilage. Osteoarthritis Cartilage 12:314-320.

Rosenberg K, Olsson H, Mörgelin M & Heinegård D (1998) Cartilage oligomeric matrix protein shows high affinity zinc-dependent interaction with triple helical collagen. J Biol Chem 273:20397-20403.

Rossi A & Superti-Furga A (2001) Mutations in the diastrophic dysplasia sulphate transporter (DTDST) gene (SLC26A2): 22 novel mutations, mutation review, associated skeletal phenotypes and diagnostic relevance. Hum Mutat 17:159-171.

Rossi A, Bonaventure J, Delezoide AL, Cetta G & Superti-Furga A (1996) Undersulfation of proteoglycans synthesized by chondrocytes from a patient with achondrogenesis type 1B for an L483P substitution in the diastrophic dysplasia sulphate transporter. J Biol Chem 271:18456-18464.

Rossi A, Kaitila I, Wilcox WR, Rimoin DL, Steinmann B, Cetta G & Superti-Furga A (1998) Proteoglycan sulfation in cartilage and cell cultures from patients with sulphate transporter chondrodysplasias: relationship to clinical severity and indications on the role of intracellular sulphate production. Matrix Biol 17:361-369.

Roughley P (2002) Articular cartilage and changes in arthritis. Noncollagenous proteins and proteoglycans in the extracellular matrix of cartilage. Arthritis Res 3:342-347.

Rousseau J-C, Farjanel J, Boutillon M-M, Hartmann DJ, van der Rest M & Moradi-Améli M (1996) Processing of type XI collagen. Determination of the matrix form of the α1(XI) chain. J Biol Chem 271:23743-23748.

Sahlman J, Pitkänen MT, Prockop DJ, Arita M, Li SW, Helminen H, Langsjö TK, Puustjärvi K & Lammi MJ (2004) A human COL2A1 gene with an Arg519Cys mutation causes osteochondrodysplasia in transgenic mice. Arthritis Rheum 50:3153-60.

Saklatvala J (1986) Tumour necrosis factor α stimulates resorption and inhibits synthesis of proteoglycan in cartilage. Nature 322:547-549.

Sambrook PN, MacGregor AJ & Spector TD (1999) Genetic influences on cervical and lumbar disc degeneration: a magnetic resonance imaging study in twins. Arthritis Rheum 42:366-72.

Sandberg M, Tamminen M, Hirvonen H, Vuorio E & Pihlajaniemi T (1989) Expression of type XIII collagen in human fetal tissues: comparison with expression of mRNAs for collagen types I, II and III. J Cell Biol 109:1371-1379.

Sandell LJ, Morris N, Robbins JR & Goldring MB (1991) Alternatively spliced type II procollagen messenger RNAs define distinct populations of cells during vertebral development – differential expression of the amino-propeptide. J Cell Biol 114:1307-19.

Satoh H, Susaki M, Shukunami C, Iyama K, Negoro T & Hiraki Y (1998) Functional analysis of diastrophic dysplasia sulphate transporter. Its involvement in growth regulation of chondrocytes mediated by sulphated proteoglycans. J Biol Chem 273:12307-12315.

Schiller A (1994) Bones and joints. In: Rubin E & Farber JL (eds) Pathology. J.B. Lippincott Company, Philadelphia, p1279-1280.

Schmid TM & Linsenmayer TF (1985) Immunohistochemical localization of short chain cartilage collagen (type X) in avian tissues. J Cell Biol 100:598-605.

Scott DA & Karniski LP (2000) Human pendrin expressed in Xenopus laevis oocytes mediates chloride/formate exchange. Am J Physiol Cell Physiol 278:C207-211.

Scott DA, Wang R, Kreman TM, Sheffield VC & Karnishki LP (1999) The Pendred syndrome gene encodes a chloride-iodide transport protein. Nat Genet 21:440-443.

Segat D, Frie C, Nitsche P, Klatt AR, Piecha D, Korpos E, Wagener R, Paulsson M & Smyth N (2000) Expression of matrilin-1, -2 and -3 in developing mouse limbs and heart. Matrix Biology 2000:649-655.

Segat D, Paulsson M & Smyth N (2001) Matrilins: structure, expression and function. Osteoarthritis Cartilage 9 SupplA:S29-S35.

Serra R, Johnson M, Filvaroff EH, LaBorde J, Sheehan DM, Derynck R & Moses HL (1997) Expression of a truncated, kinase defective TGF-β type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. J Cell Biol 139:541-552.

Shen Z, Heinegård D & Sommarin Y (1995) Distribution and expression of cartilage oligomeric matrix protein and bone sialprotein showed marked changes during rat femoral head development. Matrix Biology 14:773-781.
Shlopov BV, Lie W-R, Mainardi CL, Cole AA, Chubinskaya S & Hasty KA (1997) Osteoarthritic lesions. Involvement of three different collagenases. Arthritis Rheum 40:2065-2074.
Shum L & Nuckolls G (2002) The life cycle of chondrocytes in the developing skeleton. Arthritis Res 4:94-106.
Sirko-Osadsa A, Murray MA, Scott JA, Lavery MA, Warman ML & Robin NH (1998) Stickler syndrome without eye involvement is caused by mutations in COL11A2, the gene encoding the α2(XI) chain of type XI collagen. J Pediatr 132:368-371.
Smith AJ, Keen LJ, Billingham MJ, Perry MJ, Elson CJ, Kirwan JR, Sims JE, Doherty M, Spector TD & Bidwell JL (2004) Extended haplotypes and linkage disequilibrium in the IL1R1 and IL1A-IL1B-IL1RN gene cluster: association with knee osteoarthritis. Genes Immun 5:451-460.
Smith RKW, Zunino L, Webbon PM & Heinegård D (1997) The distribution of cartilage oligomeric matrix protein (COMP) in tendon and its variation with tendon site, age and load. Matrix Biology 16:255-271.
So CL, Kaluarachchi K, Tam PPL & Cheah KSE (2001) Impact of mutations of cartilage matrix genes on matrix structure, gene activity and chondrogenesis. Osteoarthritis Cartilage 9 SupplA:S160-S173.
Solovieva S, Lohiniva J, Leino-Arjas P, Raininko R, Luoma K, Ala-Kokko L & Riihimäki H (2002) COL9A3 gene polymorphism and obesity in intervertebral disc degeneration of the lumbar spine: evidence of gene-environment interaction. Spine 27:2692-2696.
Song HR, Lee KS, Li QW, Koo SK & Jung SC (2003) Identification of cartilage oligomeric matrix protein (COMP) gene mutations in patients with pseudoachondroplasia and multiple epiphyseal dysplasia. J Hum Genet 48:222-225.
Southam L, Dowling B, Ferreira A, Marcelline L, Mustafa Z, Chapman K, Bentham G, Carr A & Loughlin J (2004) Microsatellite association mapping of a primary osteoarthritis susceptibility locus on chromosome 6p12.3-q13. Arthritis Rheum 50:3910-3914.
Spayde EC, Joshi AP, Wilcox WR, Briggs MD, Cohn DH & Olsen BR (2000) Exon skipping mutation in the COL9A2 gene in a family with multiple epiphyseal dysplasia. Matrix Biol 19:121-129.
Spector TD & MacGregor AJ (2004) Risk factors for osteoarthritis: genetics. Ostearthritis Cartilage 12(Suppl A):S39-S44.
Spector TD, Cicuttini F, Baker J, Loughlin J & Hard D (1996) Genetic influences on osteoarthritis in women: results of a classic twin study. BMJ 312:940-943.
Stanescu R, Stanescu V, Muriel MP & Maroteaux P (1993) Multiple epiphyseal dysplasia, Fairbank type: morphologic and biochemical study of cartilage. Am J Med Genet 45:501-507.
Stankovich J, Sale MM, Cooley HM, Bahlo M, Reilly A, Dickinson JL & Jones G (2002) Investigation of chromosome 2q in osteoarthritis of the hand: no significant linkage in a Tasmanian population. Ann Rheum Dis 61:1081-1084.
Stecher RM (1941) Heberden's nodes. Heredity in hypertrophic arthritis of the finger joints. Am J Med Sci 201:801-809.
Stefánsson SE, Jónsson H, Ingvarsson T, Manolescu I, Jónsson HH, Ólafsdóttir G, Pálsdóttir E, Stefánsdóttir G, Sveinbjörnsdóttir G, Frigge ML, Kong A, Gulcher JR & Stefánsson K (2003) Genomewide scan for hand osteoarthritis: a novel mutation in matrilin-3. Am J Hum Genet 72:1448-1459.
Sugimoto M, Kimura T, Tsumaki N, Matsui Y, Nakata K, Kawahata H, Yasui N, Kitamura Y, Nomura S & Ochi T (1998) Differential in situ expression of α2(XI) collagen mRNA isoforms in the developing mouse. Cell Tissue Res 292:325-332.
Sunyaev S, Ramensky V & Bork P (2000) Towards a structural basis of human non-synonymous single nucleotide polymorphisms. Trends Genet 16:198-200.
Sunyaev S, Ramensky V, Koch I, Lathe W 3rd, Kondrashov AS & Bork P (2001) Prediction of deleterious human alleles. Hum Mol Genet 10:591-597.
Superti-Furga A (1994) A defect in the metabolic activation of sulphate in a patient with achondrogenesis type 1B. Am J Hum Genet 55:1137-1145.

Superti-Furga A, Bonafe L & Rimoin DL (2001) Molecular-pathogenetic classification of genetic disorders of the skeleton. Am J Med Genet (Semin Med Genet) 106:282-293.

Superti-Furga A, Neumann L, Riebel T, Eich G, Steinmann B, Spranger J & Kunze J (1999) Recessively inherited multiple epiphyseal dysplasia with normal stature, club foot, and double layered patella caused by a DTDST mutation. J Med Genet 36:621-624.

Susic S, McGrory J, Ahier J & Cole WG (1997) Multiple epiphyseal dysplasia and pseudoachondroplasia due to novel mutations in the calmodulin-like repeats of cartilage oligomeric matrix protein. Clin Genet 51:219-224.

Svensson L, Aszódi A, Heinegård D, Hunziker EB, Reinholt FP, Fässler R & Oldberg Å (2002) Cartilage oligomeric matrix protein-deficient mice have normal skeletal development. Mol Cell Biol 22:4366-4371.

Svensson L, Oldberg Å & Heinegård D (2001) Collagen binding proteins. Osteoarthritis Cartilage 9 Suppl A: S23-S28.

Swanson AB & Swanson GG (1985) Osteoarthritis in the hand. Clin Rheum Dis 11:393-419.

Säämänen AK, Salminen H, Dean PB, De Crombrugghe B, Vuorio EI & Metsaranta MP (2000) Osteoarthritis-like lesions in transgenic mice harbouring a small deletion mutation in type II collagen gene. Osteoarthritis Cartilage 8:248-257.

Tabor HK, Risch NJ & Myers RM (2002) Candidate-gene approaches for studying complex genetic traits: practical considerations. Nature Rev Genet 3:1-7.

Takahashi E, Hori T & Sutherland GR (1990) Mapping of the human type II collagen gene (COL2A1) proximal to fra(12) (q13.1) by nonisotopic in situ hybridization. Cytogenet Cell Genet 54:84-85.

Terwilliger JD (1995) A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic marker loci. Am J Hum Genet 56:777-787.

Tetlow LC, Adlam DJ & Woolley DE (2001) Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage. Arthritis Rheum 44:585-594.

Thom JR & Morris NP (1991) Biosynthesis and proteolytic processing of type XI collagen in embryonic chick sterna. J Biol Chem 266:7262-7269.

Thur J, Rosenberg K, Nitsche DP, Pihlajamaa T, Ala-Kokko L, Heinegård D, Paulsson M & Maurer P (2001) Mutations in cartilage oligomeric matrix protein causing pseudoachondroplasia and multiple epiphyseal dysplasia affect binding of calcium and collagen I, II and IX. J Biol Chem 276:6083-6092.

Tiller GE, Warman ML, Gong Y, Knoll JHM, Mayne R & Brewton RG (1998) Physical and linkage mapping of the gene for the alpha-3 chain of type IX collagen, COL9A3, to human chromosome 20q13.3. Cytogenet Cell Genet 81:205-207.

Trippel SB (2004) Growth factor inhibition. Potential role in the etiopathogenesis of osteoarthritis. Clin Orthop Rel Res 427 Suppl:S47-S52.

Uitterlinden AG, Burger H, Huang Q, Odding E, van Duijn C, Hofman A, Birkenhäger JC, van Leeuwen JPTM & Pols HAP (1997) Vitamin D receptor genotype is associated with radiographic osteoarthritis of the knee. J Clin Invest 100:259-263.

Uitterlinden AG, Burger H, van Duijn CM, Huang Q, Hofman A, Birkenhäger JC, van Leeuwen JPTM & Pols HAP (2000) Adjacent genes, for *COL2A1* and the vitamin D receptor, are associated with separate features of radiographic osteoarthritis of the knee. Arthritis Rheum 43:1456-1464.

Unger S & Hecht JT (2001) Pseudoachondroplasia and multiple epiphyseal dysplasia: new etiologic developments. Am J Med Genet (Semin Med Genet) 106:244-250.

Unger SL, Briggs MD, Holden P, Zabel B, Ala-Kokko L, Paassilta P, Lohiniva J, Rimoin DL, Lachman RS & Cohn DH (2001) Multiple epiphyseal dysplasia: Radiographic abnormalities correlated with genotype. Pediatr Radiol 31:10-18.

van der Hout AH, Verlind E, Beemer FA, Buys CHCM, Hofstra RMW & Scheffer H (2002) Occurrence of deletion of COL2A1 allele as the mutation in Stickler syndrome shows that a collagen type II dosage effect underlies this syndrome. Human Mutat Mutation in brief #532 (on-line).

van Mourik JB, Buma P & Wilcox WR (1998) Electron microscopical study in multiple epiphyseal dysplasia type II. Ultrastruct Pathol 22:249-251.

van Saase JLCM, van Romunde LKJ, Cats A, Vandenbroucke JP &Valkenburg HA (1989) Epidemiology of radiological osteoarthritis in a Dutch population with that in ten other populations. Ann Rheum Dis 48:271-280.

van Steensel MA, Buma P, de Waal Malefijt MC, van den Hoogen FHJ & Brunner HG (1997) Oto-spondylo-megaepiphyseal dysplasia (OSMED): Clinical description of three patients homozygous for a missense mutation in the COL11A2 gene. Am J Med Genet 70:315-323.

Vandenberg P, Khillan JS, Prockop DJ, Helminen H, Kontusaari S & Ala-Kokko L (1991) Expression of a partially deleted gene of human type II procollagen (COL2A1) in transgenic mice produces a chondrodysplasia. Proc Natl Acad Sci USA 88:7640-7644.

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

Vaughan-Thomas A, Young RD, Phillips AC & Duance VC (2001) Characterization of type XI collagen-glycosaminoglycan interactions. J Biol Chem 276:5303-5309.

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

von der Mark K & von der Mark H (1977) The role of three genetically distinct collagen types in endochondral ossification and calcification of cartilage. J Bone Joint Surg 59:458-464.

Vranka J, Mokashi A, Keene DR, Tufa S, Corson G, Sussman M, Horton WA, Maddox K, Sakai L & Bachinger HP (2001) Selective intracellular retention of extracellular matrix proteins and chaperones associated with pseudoachondroplasia. Matrix Biol 20:439-450.

Vuoristo MM, Pappas JG, Jansen V & Ala-Kokko L (2004) A stop codon mutation in COL11A2 induces exon skipping and leads to non-ocular Stickler syndrome. Am J Med Genet 130:160-164.

Vuoristo MM, Pihlajamaa T, Vandenberg P, Prockop DJ & Ala-Kokko L (1995) The human *COL11A2* gene structure indicates that the gene has not evolved with the genes for the major fibrillar collagens. J Biol Chem 270:22873-228781.

Wagener R, Kobbe B & Paulsson M (1997) Primary structure of matrilin-3, a new member of a family of extracellular matrix proteins related to cartilage matrix protein (matrilin-1) and von Willebrand factor. FEBS letters 413:129-134.

Warman ML, McCarthy MT, Perälä M, Vuorio E, Knoll JHM, McDaniels CN, Mayne R, Beier DR & Olsen BR (1994) The genes encoding alpha-2(IX) collagen (COL9A2) map to human chromosome 1q32.3-p33 and mouse chromosome 4. Genomics 23:158-162.

Warman ML, Tiller GE, Polumbo PA, Seldin MF, Rochelle JM, Knoll JHM, Cheng S-D & Olsen BR (1993) Physical and linkage mapping of the human and murine genes for the alpha-1 chain of type IX collagen (COL9A1) Genomics 17:694-698.

Weise M, De-Levi S, Barnes KM, Gafni RI, Abad V & Baron J (2001) Effects of estrogen on growth plate senescence and epihyseal fusion. Proc Natl Acad Sci USA 98:6871-6.

Werle E, Schneider C, Renner M, Volker M & Fiehn W (1994) Convenient single-step, one tube purification of PCR products for direct sequencing. Nucleic Acid Res 22:4354-4355.

Whillis J (1940) The development of synovial joints. J Anat 74:227-283.

Wiberg C, Klatt AR, Wagener R, Paulsson M, Bateman JF, Heinegård D & Mörgelin M (2003) Complexes of matrilin-1 and biglycan or decorin connect collagen VI microfibrils to both collagen II and aggrecan. J Biol Chem 278:37698-37704.

Williams CJ, Considine EL, Knowlton RG, Reginato A, Neumann G, Harrison D, Buxton P, Jimenez S & Prockop DJ (1993) Spondyloepiphyseal dysplasia and precocious osteoarthritis in a family with an Arg_{75}→Cys mutation in the procollagen type II gene (*COL2A1*) Hum Genet 92:499-505.

Williams CJ, Rock M, Considine E, McCarron S, Gow P, Ladda R, McLain D, Michels WM, Murphy W, Prockop DJ & Ganguly A (1995) Three new point mutations in type II procollagen (COL2A1) and identification of a fourth family with the COL2A1 Arg519→Cys base substitution using conformation sensitive gel electrophoresis. Hum Mol Genet 4:309-312.

Winterbottom N, Tondravi MM, Harrington TL, Klier FG, Vertel BM & Goetinck PF (1992) Cartilage matrix protein is a component of the collagen fibril of cartilage. Dev Dyn 193:266-276.

Wotton SF & Duance VC (1994) Type III collagen in normal human articular cartilage. Histochem J 26:412-416.

Wright GD, Hughes AE, Regan M & Doherty M (1996) Association of two loci on chromosome 2q with nodal osteoarthritis. Ann Rheum Dis 55:317-319.

Wu J-J & Eyre DR (1995) Structural analysis of cross-linking domains in cartilage type XI collagen. Insights on polymeric assembly. J Biol Chem 270:18865-18870.

Wu J-J & Eyre DR (1998) Matrilin-3 forms disulfide-linked oligomers with matrilin-1 in bovine epiphyseal cartilage. J Biol Chem 273:17433-17438.

Wu W, Billinghurst RC, Pidoux I, Antonious J, Zukor D, Tanzer M et al. (2002) Sites of collagenase cleavage and denaturation of type II collagen in articular cartilage in ageing and osteoarthritis and their relationship to the distribution of the collagenases MMP-1 and MMP-13. Arthritis Rheum 46:2087-2094.

Xu L, Flahiff CM, Waldman BA, Wu D, Olsen BR, Setton LA & Li Y (2003) Osteoarthritis-like changes and decreased mechanical function of articular cartilage in the joints of mice with the chondrodysplasia gene (*cho*) Arthritis Rheum 48:2509-18.

Yang X, Chen L, Xu X, Li C, Huang C & Deng C-X (2001) TGF-β/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. J Cell Biol 153:35-46.

Yoshioka SH, Inoguchi K, Khaleduzzaman M, Ninomiya Y, Andrikopoulos K & Ramirez F (1995) Coding sequence and alternative splicing of the mouse α1(XI) collagen gene (Col11a1) Genomics 28:337-340.

Young RD, Lawrence PA, Duance VC, Aigner T & Monaghan P (2000) Immunolocalization of collagen types II and III in single fibrils of human articular cartilage. J Histochem Cytochem 48:423-432.

Zaia J, Boynton RE, McIntosh A, Marshak DR, Olsson H, Heinegård D & Barry FP (1997) Post-translational modifications in cartilage oligomeric matrix protein. Characterization of the N-linked oligosaccharides by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. J Biol Chem 272:14120-14126.

Zelzer E & Olsen BR (2003) The genetic basis for skeletal diseases. Nature 423:343-348.

Zhai G, Rivadeneira F, Houwing-Duistermaat JJ, Meulenbelt I, Bijkerk C, Hofman A, van Meuers JBJ, Uitterlinden AG, Pols HAP, Slagboom PE & van Duijn CM (2004) Insulin-like growth factor 1 gene promoter polymorphism, collagen type II α1 (COL2A1) gene, and the prevalence of radiographic osteoarthritis: the Rotterdam study. Ann Rheum Dis 63:544-548.

Zhang Y & Chen Q (2000) Changes of matrilin forms during endochondral ossification. Molecular basis of oligomeric assembly. J Biol Chem 275:32628-32634.

Zheng J, Shen W, He DZ, Long KB, Madison LD & Dallos P (2000) Prestin is the motor protein of cochlear outer hair cells. Nature 405:149-155.

Zhidkova NI, Justice SK & Mayne R (1995) Alternative mRNA processing occurs in the variable region of the pro-α1(XI) and pro-α2(XI) collagen chains. J Biol Chem 270:9486-9493.

Zhu Y, Oganesian A, Keene DR & Sandell LJ (1998) Type IIA procollagen containing the cysteine-rich amino propeptide is deposited in the extracellular matrix of prechondrogenic tissue and binds to TGF-β1 and BMP-2. J Cell Biol 144:1069-1080.

Özbek S, Engel J & Stetefeld J (2002) Storage function of cartilage oligomeric matrix protein: the crystal structure of the coiled-coil domain in complex with vitamin D_3. EMBO J 21:5960-5968.

Appendix

Supplemental table 1. Association studies of candidate genes with hand, hip and/or knee OA

Gene	Variation(s) tested	Site of OA*	Sex	Dg	n of cases/ controls	Country	Association ($p<0.05$)	OR (95 % CI)	Reference
Cartilage ECM related genes									
COL2A1	BamHI	GOA	F	ROA	86/91	UK	p 0.04		Hull & Pope 1989
	BamHI, PvuII, HindIII	GOA	NA	ROA	60/37-167	UK	No		Priestley et al. 1991
	PvuII, VNTR	Ha/GOA	NA	ROA	49+41/48	Finnish	No		Vikkula et al. 1993
	c.3268G>A/ MaeII	GOA	F+M	NA	76/73	UK	p 0.0047	NA	Loughlin et al. 1995
	HaeIII, HindIII, MaeII, VNTR	GOA	F+M	ROA	123/697	Netherlands	No		Meulenbelt et al. 1999
	HT of HaeIII, HindIII, VNTR	GOA	F+M	ROA	123/697	Netherlands	p 0.0083	5.3 (2.2-12.7)	Meulenbelt et al. 1999
	VNTR (13R1 allele)	Ha/H/K/S	F+M	ROA	191/1355	Netherlands	No		Zhai et al. 2004
	VNTR	K	F+M	ROA	183/668p	Netherlands	Yes	2.06 (1.27-3.34)	Uitterlinden et al. 2000
	HaeIII, G565G, G191S	K/H	F>M	ROA	417/280	Japan	No		Ikeda et al. 2002
	HT of SNPs (e5, e32, e51)	K/H	F>M	ROA	417/280	Japan	p 0.024	1.30 (1.04-1.63)	Ikeda et al. 2002
	PvuII	H	F	THR	75/239	Belgium	No		Aerssens et al. 1998
	PvuII	H	NA	THRa	101+42/50	Italy	No		Granchi et al. 2002
COL9A1	c.2271G>A, P757P	K/H	F>M	ROA	417/280	Japan	No		Ikeda et al. 2002
	20 SNPs	H	F	THR	146/215	UK	No		Loughlin et al. 2002a
COL9A2	Q326R, Q326T	K/H	F>M	ROA	417/280	Japan	No		Ikeda et al. 2002
COL9A3	c.1740C>T, P580P	K/H	F>M	ROA	417/280	Japan	p 0.015	1.31 (1.05-1.63)	Ikeda et al. 2002
COL11A1	c.4770C>T, I1590I	K/H	F>M	ROA	417/280	Japan	No		Ikeda et al. 2002
COL11A2	Q226K, P1058P, P1128P	K/H	F>M	ROA	417/280	Japan	No		Ikeda et al. 2002
ADLICAN	C>T, G2663D	K	F	ROA	280/469p	UK	No		Valdes et al. 2004

Gene	Variation(s) tested	Site of OA*	Sex	Dg	n of cases/ controls	Country	Association (p<0.05)	OR (95 % CI)	Reference
AGC1	VNTR	Ha	M	ROA	43/50	USA	p<0.05 (a27)	3.23 (1.24-8.41)adj: age, BMI	Horton et al. 1998
	VNTR	K	M	ROA	28/65	USA	No		Horton et al. 1998
	VNTR	Ha/H/K	NA	ROA	n = 134t	Australia	No	NA	Kirk et al. 2003
ASPN	8 polymorphisms	K	F>M	ROA	530/608	Japan	p 0.00024 (D14)	1.87 (1.3-2.6)	Kizawa et al. 2005
	8 polymorphisms	K	F>M	ROA	593/374	Japan	p 0.0078 (D14)	1.70 (1.1-2.5)	Kizawa et al. 2005
BGN	G>T	K	F	ROA	280/469p	UK	No		Valdes et al. 2004
CILP	C>T, T395I	K	F	ROA	280/469p	UK	p 0.021		Valdes et al. 2004
COMP	6 polymorphisms	K/H	NA	ROA	402/2282	Japan	No		Mabuchi et al. 2001
	A>G, N386D	K/H	F	ROA	280/469p	UK	No		Valdes et al. 2004
CRTL1	CA-repeat	K/H	F+M	ROA	126/135	Netherlands	No		Meulenbelt et al. 1997
	CA-repeat	H	F+M	ROA	54/135	Netherlands	No		Meulenbelt et al. 1997
DTDST	3 polymorphisms	K	F>M	ROA	165/127	Japan	p 0.043 (del4A)	3.43 (0.97-12.17)	Ikeda et al. 2001
MATN1	microsatellite	K	F+M	ROA	126/135	Netherlands	No		Meulenbelt et al. 1997
	microsatellite	H	F+M	ROA	54/135	Netherlands	p 0.04 (males)	0.50 (0.26-0.95)adj: age, BMI	Meulenbelt et al. 1997
	microsatellite	K/H	F+M	TJR	338/338	UK	No		Loughlin et al. 2000a
	microsatellite	GOA/H/K/S	F>M	ROA	73/53	Argentina	No		Strusberg et al. 2002
MATN3	5 SNPs	Ha	F+M	ROA	2612/873	Iceland	Yes	RR 2.12 (0.92-4.86)	Stefansson et al. 2003
PAPSS2	5 SNPs	K	F>M	ROA	165/127	Japan	No		Ikeda et al. 2001
Bone related genes									
COL1A1	G>T/AccB71	H	F	THR	75/239	Belgium	No		Aerssens et al. 1998

Gene	Variation(s) tested	Site of OA*	Sex	Dg	n of cases/ controls	Country	Association (p<0.05)	OR (95 % CI)	Reference
	BalI/MscI	K/H	F+M	TJR	371/369	UK	No		Loughlin et al. 2000b
VDR	T>C, I365I/TaqI	K	F	ROA	82/269p	UK	p <0.05	2.60 (1.01-6.71)adj: age, BMI, BMD,HRT	Keen et al. 1997
	T>C, I365I/TaqI	K	F	ROA	280/469p	UK	No		Valdes et al. 2004
	HT of BsmI, ApaI, TaqI	K	F+M	ROA	179/667p	Netherlands	p 0.005	2.31 (1.48-3.59)adj: age, BMI, BMD	Uitterlinden et al. 1997, 2000
	BsmI, ApaI, TaqI + HT	K	F	ROA	167/105	Japan	No		Huang et al. 2000
	T>C, I365I/TaqI	K/H	F+M	TJR	371/369	UK	No		Loughlin et al. 2000b
	BsmI	H	F	THR	75/239	Belgium	No		Aerssens et al. 1998
	BsmI, ApaI, TaqI + HT	H	F	ROA	146/158	Japan	No		Huang et al. 2000
	BsmI, ApaI, TaqI + HT	Ha	F	ROA	134/127	Japan	No		Huang et al. 2000
	BsmI, ApaI, TaqI + HT	GOA	F	ROA	46/88	Japan	No		Huang et al. 2000
BMP2	S87S	K	F	ROA	280/469p	UK	p 0.007	1.68 (1.15-2.46)	Valdes et al.
	S190R	K	F	ROA	280/469p	UK	p 0.005	1.72 (1.18-2.52)	2004
									Valdes et al. 2004
BMP5	8 polymorphisms	H	F	THR	146/215	UK	No		Southam et al. 2003

Gene	Variation(s) tested	Site of OA*	Sex	Dg	n of cases/ controls	Country	Association (p<0.05)	OR (95 % CI)	Reference
BMPR1A	del>ins	K	F	ROA	280/469p	UK	No		Valdes et al. 2004
OGN	A>G	K	F	ROA	280/469p	UK	No		Valdes et al. 2004
Inflammatory system related genes									
IL1A	-889C>T / NcoI	K/H	F+M	TJR	557/557	UK	p 0.04	1.5 (1.0-2.1)	Loughlin et al. 2002b
	-889C>T	Ha	F>M	ROA	68/51	US	No		Stern et al. 2003
IL1B	-511C>T / AvaI	K	F+M	ROA	139/747p	Netherlands	No		Meulenbelt et al. 2004
	+3954C>T / TaqI	K	F+M	ROA	139/747p	Netherlands	No		Meulenbelt et al. 2004
	-511C>T / AvaI	K/H	F+M	TJR	557/557	UK	No		Loughlin et al. 2002b
	+3954C>T / TaqI	K/H	F+M	TJR	557/557	UK	No		Loughlin et al. 2002b
	+3954C>T / TaqI	K/H	NA	TJR	61/254	Germany	p 0.0096	2.59 (1.4-4.7)	Moos et al. 2000
	5810G>A / BstUI	K/H	F+M	TJR	557/557	UK	No		Loughlin et al. 2002b
	-511C>T / AvaI	H	F+M	ROA	70/816p	Netherlands	p 0.004	1.5 (0.8-2.9) adj :sex, age, BMI, BMD	Meulenbelt et al. 2004
	+3954C>T / TaqI	H	F+M	ROA	70/816p	Netherlands	p 0.003	0.6 (0.4-1.2)adj :sex, age, BMI, BMD	Meulenbelt et al. 2004
	1903T>C	Ha	F>M	ROA	68/51	USA	No		Stern et al. 2003
	-511C>T	Ha	F>M	ROA	68/51	USA	No		Stern et al. 2003
	-511C>T	Ha	F+M	ROA	38/848p	Netherlands	No		Meulenbelt et al. 2004
	5810G>A	Ha	F>M	ROA	68/51	USA	p 0.021 (AA)	RR 3.82 (NA)	Stern et al. 2003
	+3954C>T / TaqI	Ha	F+M	ROA	38/848p	Netherlands	No		Meulenbelt et al. 2004
	5887C>T	Ha	F>M	ROA	68/51	USA	No		Stern et al. 2003
IL1RN	VNTR	K	F+M	ROA	139/747p	Netherlands	No		Meulenbelt et al. 2004

Gene	Variation(s) tested	Site of OA*	Sex	Dg	n of cases/ controls	Country	Association (p<0.05)	OR (95 % CI)	Reference
	VNTR	H	F+M	ROA	70/816p	Netherlands	p 0.001 (a2)	1.7 (OR 0.9-3.1) adj: sex, age, BMI, BMD	Meulenbelt et al. 2004
	VNTR	K/H	NA	TJR	61/254	Germany	No		Moos et al. 2000
	9589A>T / SspI	K/H	F+M	TJR	557/557	UK	No		Loughlin et al. 2002b
	11100T>C / MspA1I	K/H	F+M	TJR	557/557	UK	No		Loughlin et al. 2002b
	8006T>C	Ha	F>M	ROA	68/51	USA	No		Stern et al. 2003
	VNTR	Ha	F>M	ROA	68/51	USA	No		Stern et al. 2003
	VNTR	Ha	F+M	ROA	38/848p	Netherlands	p 0.044 (a2)	NA	Meulenbelt et al. 2004
	11100T>C	Ha	F>M	ROA	68/51	USA	No		Stern et al. 2003
IL1A/IL1RN	HT of IL1A-889 / IL1RN 9589	K/H	F+M	TJR	557/557	UK	p 0.009	1.7 (1.2-2.4)	Loughlin et al. 2002b
IL4R	C>T, N142N	K/H	F	TJR	456/399	UK	p 0.03	1.5 (1.1-2.1)	Forster et al. 2004
	C>T, S411L	K/H	F	TJR	456/399	UK	p 0.01	2.0 (1.2-3.3)	Forster et al. 2004
	T>G, S727A	K/H	F	TJR	456/399	UK	p 0.004	2.1 (1.3-3.5)	Forster et al. 2004
IL6	-174G>C	K/H	NA	TJR	61/254	Germany	No		Moos et al. 2000
IL8	3 SNPs	H	F	THR	133/375	UK	No		Kawahara et al. 2005
TNFα	-308G>A	K/H	NA	TJR	61/254	Germany	No		Moos et al. 2000
Other genes									
Erα	PvuII + XbaI (PpXx)	GOA	F	ROA	65/318	Japan	p 0.039	1.86 (1.03-3.24)	Ushiyama et al. 1998
	PvuII, XbaI	K/H	F+M	TJR	371/369	UK	No		Loughlin et al. 2000b
	HT of PvuII + XbaI	K	F+M	ROA	361/1122[p]	Netherlands	p <0.01	1.3 (0.9-1.7)[adj: age, BMI, BMD, smoking,, MP]	Bergink et al. 2003
	T>C/PvuII	K	F	ROA	280/469[p]	UK	No		Valdes et al. 2004
FRZB	e4+6C>T, R200W	H	F+M	THR	936/760	UK	No		Loughlin et al. 2004
	e6+109C>G, R324W	H	F+M	THR	936/760	UK	p 0.02 (female)	1.5 (1.1-2.1)	Loughlin et al. 2004

Gene	Variation(s) tested	Site of OA*	Sex	Dg	n of cases/ controls	Country	Association (p<0.05)	OR (95 % CI)	Reference
	HT of e4 and e6 SNPs	H	F+M	THR	936/760	UK	p 0.007 (female)	4.1 (1.6-10.7)	Loughlin et al. 2004
IGF-1	CA-repeat	Ha/H/K/S	F+M	ROA	651/135[p]	Netherlands	p 0.02 (A3)	1.9 (1.2-3.1)[adj: sex, age, BMI, BMD]	Meulenbelt et al. 1998
	CA-repeat	Ha/H/K/S	F+M	ROA	1355/191[p]	Netherlands	p for trend 0.03	1.4 (1.0-1.8)[adj: sex, age, BMI, BMD]	Zhai et al. 2004
IGFBP7	IVS2+T>A	H	F	THR	144/375	UK	No		Kawahara et al. 2005
A1AT	RFLP	GOA	NA	ROA	35/125	UK	No		Sakkas et al. 1990
A1ACT	TaqI	GOA	NA	ROA	35/125	UK	p (0.02)	RR 2.2	Sakkas et al. 1990
AACT	2 SNPs	K	F	ROA	280/469[p]	UK	p 0.013 (A^9T)		Valdes et al. 2004
ACLP	C>T	K	F	ROA	280/469[p]	UK	No		Valdes et al. 2004
ADAM12	G>C, G48R	K	F	ROA	280/469[p]	UK	p 0.004	1.84 (1.22-2.79)	Valdes et al. 2004
ADAMTS3	6 polymorphisms	H	F	THR	144-388/375	UK	No		Kawahara et al. 2005
CD36	-120 A>C	K	F	ROA	280/469[p]	UK	p 0.020	0.77 (0.61-0.96)	Valdes et al. 2004
COX2	G>C, V102V	K	F	ROA	280/469[p]	UK	p 0.027	0.66 (0.46-0.95)	Valdes et al. 2004
CTSL	C>T	K	F	ROA	280/469[p]	UK	No		Valdes et al. 2004
DAF	A>G	K	F	ROA	280/469p	UK	No		Valdes et al. 2004
IBSP	A>G, G195E	K	F	ROA	280/469p	UK	No		Valdes et al. 2004
HFE	H63D	Ha	F>M	ROA	176/2138	USA	No		Ross et al. 2003
	C282Y	Ha	F>M	ROA	176/2138	USA	p 0.029	NA	Ross et al. 2003
MMP-3	C>T	K	F	ROA	280/469p	UK	No		Valdes et al. 2004
NCOR2	A>G, T1699A	K	F	ROA	280/469p	UK	p 0.015	1.57 (1.09-2.25)	Valdes et al. 2004
OPG	C>T	K	F	ROA	280/469p	UK	p 0.047 (progr)		Valdes et al. 2004
SOD3	C>T	K	F	ROA	280/469p	UK	No		Valdes et al. 2004

Gene	Variation(s) tested	Site of OA*	Sex	Dg	n of cases/ controls	Country	Association (p<0.05)	OR (95 % CI)	Reference
TIMP1	C>T, L124L	K	F	ROA	280/469p	UK	No		Valdes et al. 2004
TNA	A>G, S106G	K	F	ROA	280/469p	UK	p 0.014 (progr)		Valdes et al. 2004
TNFAIP6	e4+37G>A, R144Q	K	F	ROA	280/469p	UK	No		Valdes et al. 2004
	e4+37G>A, R144Q	H	F+M	THR	378/760	UK	No		Loughlin et al. 2004

* Ha = hand; H = hip; K = knee; S = spine

HT = haplotype; VNTR = variable number of tandem repeats

ROA = radiological OA; THR = total hip replacement; TJR = total joint replacement, either THR or TKR; TKR = total knee replacement

p = population based study

progr = progression of OA

a Either patients with primary hip OA patients or patients with secondary OA due to hip dysplasia

adj = adjusted for, BMI = body mass index; BMD = bone mineral density; HRT = use of hormone replacement therapy; MP = age at menopause

References for the supplemental table 1.

Aerssens J, Dequeker J, Peeters J, Breemans S & Boonen S (1998) Lack of association between osteoarthritis of the hip and gene polymorphisms of *VDR*, *COL1A1*, and *COL2A1* in postmenopausal women. Arthritis Rheum 41:1946-1950.

Bergink AP, van Meuers JB, Loughlin J, Arp PP, Fang Y, Hofman A, van Leeuwen JPTM, van Duijn CM, Uitterlinden AG & Pols HAP (2003) Estrogen receptor α gene haplotype is associated with radiographic osteoarthritis of the knee in elderly men and women. Arthritis Rheum 48:1913-1922.

Forster T, Chapman K & Loughlin J (2004) Common variants within the interleukin 4 receptor α gene (IL4R) are associated with susceptibility to osteoarthritis. Hum Genet 114:391-395.

Granchi D, Stea S, Sudanese A, Toni A, Baldini & Giunti A (2002) Association of two gene polymorphisms with osteoarthritis secondary to hip dysplasia. Clin Orthop 403:108-117.

Horton WE, Lethbridge-Cejku M, Hochberg MC, Balakir R, Precht P, Plato CC, Tobin JD, Meer L & Doege K (1998) An association between an aggrecan polymorphic allele and bilateral hand osteoarthritis in elderly white men: data from the Baltimore Longitudinal Study of Aging (BLSA). Osteoarthritis Cartilage 6:245-251.

Huanq J, Ushiyama T, Inoue K, Kawasaki T & Hukuda S (2000) Vitamin D receptor gene polymorphisms and osteoarthritis of the hand, hip, and knee: a case-control study in Japan. Rheumatol 2000 39:79-84.

Hull & Pope (1989) Osteoarthritis and cartilage collagen genes. Lancet 8650:1337-1338.

Ikeda T, Mabuchi A, Fukuda A, Hiraoka H, Kawakami A, Yamamoto S, Machida H, Takatori Y, Kawaguchi H, Nakamura K & Igekawa S (2001) Identification of sequence polymorphisms in two sulfation-related genes, PAPSS2 and SLC26A2, and an association analysis with knee osteoarthritis. J Hum Genet 46:538-543.

Ikeda T, Mabuchi A, Fukuda A, Kawakami A, Yamada R, Yamamoto S, Miyoshi K, Haga N, Hiraoka H, Takatori Y, Kawaguchi H, Nakamura K & Igekawa S (2002) Association analysis of single nucleotide polymorphisms in cartilage-specific collagen genes with knee and hip osteoarthritis in the Japanese population. J Bone Miner Res 17:1290-1296.

Kawahara C, Forster T, Chapman K, Carr A & Loughlin J (2005) Genetic association analysis of the IGFBP7, ADAMTS3, and IL8 genes as the potential osteoarthritis susceptibility that maps to chromosome 4. Ann Rheum Dis 64:474-476.

Keen RW, Hart DJ, Lanchbury JS & Spector TD (1997) Association of early osteoarthritis of the knee with a *Taq* I polymorphism of the vitamin D receptor gene. Arthritis Rheum 40:1444-1449.

Kirk KM, Doege KJ, Hecht JT, Bellamy N & Martin NG (2003) Osteoarthritis of the hands, hips and knees in an Australian twin sample – evidence of association with Aggrecan VNTR polymorphism. Twin Res 6:62-66.

Kizawa H, Kou I, Iida A, Sudo A, Miyamoto Y, Fukuda A, Mabuchi A, Kotani A, Kawakami A, Yamamoto S, Uchida A, Nakamura K, Notoya K, Nakamura Y & Igekawa S (2005). An aspartic acid repeat polymorphism in asporin inhibits chondrogenesis and increases susceptibility to osteoarthritis. Nat Genet, in press (published on-line 9.1.2005).

Loughlin J, Dowling B, Chapman K, Marcelline L, Mustafa Z, Southam L, Ferreira A, Ciesielski C, Carson DA & Corr M (2004) Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. Proc Natl Acad Sci USA 101:9757-9762.

Loughlin J, Dowling B, Mustafa Z & Chapman K (2002b) Association of the interleukin-1 gene cluster on chromosome 2q13 with knee osteoarthritis. Arthritis Rheum 46:1519-1527.

Loughlin J, Dowling B, Mustafa Z, Smith A, Sykes B & Chapman K (2000a) Analysis of the association of the matrilin-1 gene (CRTM) with osteoarthritis: comment on the article by Meulenbelt et al. Arthritis Rheum 43:1423-5.

Loughlin J, Irven C, Athanasou N, Carr A & Sykes B (1995) Differential allelic expression of the type II collagen gene (COL2A1) in osteoarthritic cartilage. Am J Hum Genet 56:1186-1193.

Loughlin J, Mustafa Z, Dowling B, Southam L, Marcelline L, Räinä SS, Ala-Kokko L & Chapman K (2002a) Finer linkage mapping of a primary hip osteoarthritis susceptibility locus on chromosome 6. Eur J Hum Genet 10:562-568.

Loughlin J, Sinsheimer JS, Mustafa Z, Carr AJ, Clipsham K, Bloomfield VA, Chitnavis J, Bailey A, Sykes B & Chapman K (2000b) Association analysis of the vitamin D receptor gene, the type I collagen gene COL1A1, and the estrogen receptor gene in idiopathic osteoarthritis. J Rheumatol 27:779-784.

Mabuchi A, Ikeda T, Fukuda A, Koshizuka Y, Hiraoka H, Miyoshi K, Haga N, Kawaguchi H, Kawakami A, Yamamoto S, Takatori Y, Nakamura K & Igekawa S (2001) Identification of sequence polymorphisms of the *COMP* (cartilage oligomeric matrix protein) gene and association study in osteoarthrosis of the knee and hip joints. J Hum Genet 46:456-462.

Meulenbelt I, Bijkerk C, de Wildt SC, Miedema HS, Valkenburg HA, Breedveld FC, Pols HA, Te Koppele JM, Sloos VF, Hofman A, Slagboom PE & van Duijn CM (1997) Investigation of the association of the CRTM and CRTL1 genes with radiographically evident osteoarthritis in subjects from the Rotterdam study. Arthritis Rheum 40:1760-1765.

Meulenbelt I, Bijkerk C, de Wildt SCM, Miedema HS, Breedveld FC, Pols HAP, Hofman A, van Duijn CM & Slagboom PE (1999) Haplotype analysis of three polymorphisms of the COL2A1 gene and associations with generalised radiological osteoarthritis. Ann Hum Genet 63:393-400.

Meulenbelt I, Bijkerk C, Miedema HS, Breedveld FC, Hofman A, Valkenburg HA, Pols HAP, Slagboom PE & van Duijn CM (1998) A genetic association study of the IGF-1 gene and radiological osteoarthritis in a population-based cohort study (the Rotterdam study). Ann Rheum Dis 57:371-374.

Meulenbelt I, Seymour AB, Nieuwland M, Huizinga TWJ, van Duijn CM & Slagboom PE (2004) Association of the interleukin-1 gene cluster with radiographic signs of osteoarthritis of the hip. Arthritis Rheum 50:1179-1186.

Moos V, Rudwaleit M, Herzog V, Höhlig K, Sieper J & Müller B (2000) Association of genotypes affecting the expression of interleukin-1β or interleukin-1 receptor antagonist with osteoarthritis. Arthritis Rheum 43:2417-2422.

Priestley L, Fergusson C, Ogilvie D, Wordsworth P, Smith R, Pattrick M, Doherty M & Sykes B (1991). A limited association of generalized osteoarthritis with alleles at the type II collagen locus: COL2A1. Br J Rheumatol 30:272-275.

Ross J, Kowalchuk RM, Shaulinsky J, Ross L, Ryan D & Phatak PD (2003) Association of heterozygous hemochromatosis C282Y gene mutation with hand osteoarthritis. J Rheumatol 30:121-125.

Sakkas LI, Macfarlane DG, Bird H, Welsh KI & Panayi GS (1990) Association of osteoarthritis with homozygosity for a 5.8 KB TaqI fragment of the α1-antichymotrypsin gene. Br J Rheumatol 29:245-248.

Southam L, Chapman K & Loughlin J (2003) Genetic association analysis of BMP5 as a potential osteoarthritis susceptibility gene. Rheumatol 42:911-912

Stefánsson SE, Jónsson H, Ingvarsson T, Manolescu I, Jónsson HH, Ólafsdóttir G, Pálsdóttir E, Stefánsdóttir G, Sveinbjörnsdóttir G, Frigge ML, Kong A, Gulcher JR & Stefánsson K (2003) Genomewide scan for hand osteoarthritis: a novel mutation in matrilin-3. Am J Hum Genet 72:1448-1459.

Stern AG, de Carvalho MRC, Buck GA, Adler RA, Rao TPS, Disler D, Moxley G & members of the I-NODAL network (2003) Association of erosive hand osteoarthritis with a single nucleotide polymorphism on the gene encoding interleukin-1 beta. Osteoarthritis Cartilage 11:394-402.

Strusberg I, Sembaj A, Tabares S, Strusberg AM, del Castillo I, Villamar M & Moreno Barral J (2002) Association analysis of genotypic frequencies of matrilin-1 gene in patients with osteoarthritis. Clin Exp Rheumatol 20:543-545.

Uitterlinden AG, Burger H, Huanq Q, Odding E, van Duijn C, Hofman A, Birkenhäger JC, van Leeuwen JPTM & Pols HAP (1997) Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J Clin Invest 100:259-263.

Uitterlinden AG, Burger H, van Duijn CM, Huang Q, Hofman A, Birkenhager JC, van Leeuwen JP & Pols HAP (2000) Adjacent genes, for COL2A1 and the vitamin D receptor, are associated with separate features of radiographic osteoarthritis of the knee. Arthritis Rheum 43:1456-1464.

Ushiyama T, Ueyama H, Inoue K, Nishioka J, Ohkubo I & Hukuda S (1998) Estrogen receptor gene polymorphism and generalized osteoarthritis. J Rheumatol 25:134-137.

Valdes AM, Hart DJ, Jones KA, Surdulescu G, Swarbrick P, Doyle DV, Schafer AJ & Spector TD (2004) Association study of candidate genes for the prevalence and progression of knee osteoarthritis. Arthritis Rheum 50:2497-2507.

Vikkula M, Nissila M, Hirvensalo E, Nuotio P, Palotie A, Aho K & Peltonen L (1993). Multiallelic polymorphism of the cartilage collagen gene: no association with osteoarthrosis. Ann Rheum Dis 52:762-764.

Zhai G, Rivadeneira F, Houwing-Duistermaat JJ, Meulenbelt I, Bijkerk C, Hofman A, van Meurs JBJ, Uitterlinden AG, Pols HAP, Slagboom PE & van Duijn CM (2004) Insulin-like growth factor I gene promoter polymorphism, collagen type II α1 (COL2A1) gene, and the prevalence of radiographic osteoarthritis: the Rotterdam study. Ann Rheum Dis 63:544-548.

Supplemental table 2. Candidate genes analysed in OA association studies, chromosomal location of these genes and proteins encoded by them

Gene	Polypeptide/protein	Chromosomal location	Gene bank accession
Cartilage ECM related genes			
COL2A1	α1 chain type II collagen	12q12-q13.1	NM_033150
COL9A1	α1 chain type IX collagen	6q12-q13	NM_001851
COL9A2	α2 chain type IX collagen	1p32	NM_001852
COL9A3	α3 chain type IX collagen	20q13.3	NM_001853
COL11A1	α1 chain type XI collagen	1p21	NM_001854
COL11A2	α2 chain type XI collagen	6p21.3	NM_080680
ADLICAN	Adhesion protein with leucine-rich repeats and immunoglobulin domains related to perlecan	Xp22.33	NM_015419
AGC1	Aggrecan	15q26	NM_001135
ASPN	Asporin	9q21.3-q22	NM_017680
BGN	Biglycan	Xq22	NM_001711
CILP	Cartilage intermediate-layer protein	15q22	NM_003613
COMP	Cartilage oligomeric matrix protein	19p13.1	NM_000095
CRTL1	Cartilage link protein	5q13-q14.1	NM_001884
DTDST (SLC26A2)	Diastrophic dysplasia sulphate transporter	5q31-q34	NM_000112
MATN1 (CRTM)	Matrillin-1	1p35	NM_002379
MATN3	Matrillin-3	2p24-p23	NM_002381
PAPSS2	3'-phosphoadenosine 5'-phosphosulphate synthase 2	10q22-q24	NM_004670

Gene	Polypeptide/protein	Chromosomal location	Gene bank accession
Bone related genes			
COL1A1	α1 chain type I collagen	17q21.3-q22	NM_000088
VDR	Vitamin D receptor	12q13.1	NM_000376
BMP2	Bone morphogenetic protein 2	20p12	NM_001200
BMP5	Bone morphogenetic protein 5	6p12.1	NM_021073
BMPR1A	Bone morphogenetic protein receptor; type 1A	10q22.3	NM_004329
OGN	Osteoglycin (osteoinductive factor)	9q22	NM_033014
Inflammatory system related genes			
IL1A	Interleukin-1 α	2q13	NM_000575
IL1B	Interleukin-1 β	2q14	NM_000576
IL1RN	IL-1 receptor antagonist	2q14.2	NM_173842
IL4R	Interleukin 4 receptor α	16p12.1	NM_000418
IL6	Interleukin-6	7p21	NM_000600
IL8	Interleukin-8	4q13.3	NM_000584
TNFa	Tumor necrosis factor α	6p21.3	NM_000594
Other genes			
ERa (ESR)	Estrogen receptor α	6q22.3-q23.1	NM_000125
FRZB	Secreted frizzled-related protein3	2qter	NM_001463
IGF-1	Insulin-like growth factor-1	12q21.3	NM_000618
IGFBP7	Insulin-like growth factor binding protein-7	4q12	NM_001553
A1AT	α1-antitrypsin	14q32.1	NM_000295
A1ACT	α1-antichymotrypsin	14q32.1	
AACT	Serine proteinase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 3	14q32.1	NM_001085
ACLP (AEB1)	AE binding protein 1	7p13	NM_001129
ADAM12	A disintegrin and metalloproteinase domain 12 (meltrin α)	10q26.3	NM_003474
ADAMTS3	A disintegrin and metalloproteinase domain with thrombospondin motifs 3	4q13.3	NM_014243
CD36	CD36 antigen (type I collagen receptor; thrombospondin receptor)	17q11.2	NM_001001548

Gene	Polypeptide/protein	Chromosomal location	Gene bank accession
COX2 (PTGS2)	Prostaglandin-endoperoxide synthase 2	1q25	NM_000963
CTSL	Cathepsin L	9q21-q22	NM_001912
DAF	Decay-accelerating factor for complement (CD55)	1q32	NM_000574
IBSP	Integrin-binding sialoprotein (bone sialoprotein)	4q21-q25	NM_004967
HFE	Hemochromatosis gene	6p21.3	NM_000410
MMP-3	Matrix metalloproteinase-3	11q22.3	NM_002422
NCOR2	Nuclear receptor corepressor 2	12q24	NM_006312
OPG	Tumor necrosis factor superfamily, member11b (osteoprogerin)	8q24	NM_002546
SOD3	Superoxide dismutase 3, extracellular	4p16	NM_003102
TIMP1	Tissue inhibitor of metalloproteinase 1	Xp11.3-p11.23	NM_003254
TNA (CLEC3B)	Tetranectin (plasminogen-binding protein)	3p22-p21.3	NM_003278
TNFAIP6	Tumor necrosis factor α-induced protein 6	2q23.3	NM_007115