Olli-Pekka Kämäräinen

THE SEARCH FOR SUSCEPTIBILITY GENES IN OSTEOARTHRITIS
OLLI-PEKKA KÄMÄRÄINEN

THE SEARCH FOR SUSCEPTIBILITY GENES IN OSTEOARTHRITIS

Academic dissertation to be presented with the assent of the Faculty of Medicine of the University of Oulu for public defence in Auditorium 101 A of the Faculty of Medicine (Aapistie 5 A), on 11 June 2009, at 12 noon

OULUN YLIOPISTO, OULU 2009
Abstract

This work engaged Finnish females affected with osteoarthritis (OA) of the hand to define the role of common sequence variations within the genes of the important structural protein of cartilage, aggrecan (AGC1), and the genes of inflammatory mediators, the interleukin 1 gene cluster and interleukin 6 (IL6), as possible risk factors for the disease. Also, a genome-wide linkage analysis was performed in a sample consisting of Finnish families with multiple individuals affected with hip and knee OA in order to reveal new chromosomal areas that are likely to contain disease associated variations.

OA is a chronic disease that leads to the degeneration of articular cartilage in synovial joints. The etiology of the disease is for the most part unknown. Joints of the hand, hip and knee are most commonly affected, and obesity, trauma and excess mechanical stress are known risk factors for the disease. OA also has a significant genetic component.

AGC1 carries a variable number of tandem repeats (VNTR) polymorphism, which may be significant for the biomechanical properties of cartilage. It was shown that the most common allele with 27 tandem repeats is protective against hand OA (HOA) (odds ratio 0.46, 95% confidence interval 0.27–0.78). Also, carrying two copies of any of the shorter or longer alleles increased the risk of the disease.

Inflammation seems to play a role in the etiology of OA and certain polymorphisms within the interleukin 1 gene cluster and IL6 have been previously shown to increase the transcription of these molecules and to associate with OA. In this study it was shown that the G alleles in three common IL6 promoter single nucleotide polymorphism (SNP) sites are associated with the risk of more severe forms of HOA (p = 0.001 for GGG haplotype). A SNP in IL1B associated with the bilateral form of the disease (p = 0.006) and two IL1B-IL1RN extended haplotype alleles were associated with the same phenotype.

Genome-wide and fine mapping linkage analyses recognized chromosomal locus 2q21 with a multipoint LOD score of 3.96. Despite the association analyses of several candidate genes within the locus, no disease-associating sequence variants were identified.

Keywords: inflammation, linkage analysis, Osteoarthritis - genetics
To my family
Acknowledgements

This study was carried out at the Oulu Center for Cell-Matrix Research, at the Department of Medical Biochemistry and Molecular Biology, University of Oulu, during the years 2003–2009.

I am deeply grateful to my supervisor, Docent Minna Männikkö, for guiding me through this work with her expert advice, constant support and never-ending optimism. I wish to thank her for all the time and effort she has put into my work and for being encouraging throughout these years. Professor Leena Ala-Kokko deserves my warmest appreciation for introducing me to the world of medical science.

I am thankful for the excellent facilities and scientific atmosphere created by Professors Taina Pihlajaniemi, Johanna Myllyharju, Seppo Vainio, Docent Peppi Karppinen and Research Professors Emeritus Kari Kivirikko and Ilmo Hassinen. Docents Jari Arokoski and Janna Saarela are acknowledged for their valuable comments on the thesis. Sandra Hänninen, M.Sc., deserves thanks for the careful revision of the language of the thesis.

I would like to thank Docent Päivi Leino-Arjas and Svetlana Solovieva, Ph.D., for their opinions and essential contributions in preparing the original articles. Svetlana Solovieva is especially acknowledged for her extraordinary statistical work. I appreciate the opportunity of working with Eveliina Jakkula, M.D., Ph.D., and Mari Taipale, M.Sc.. Other collaborators and research group members, both past and present, are also acknowledged.

I am grateful to Aira Harju, Minta Lumme, Helena Satulehto and Irma Vuoti for their expert assistance in the laboratory. Special thanks for the valuable help with practical matters go to Pertti Vuokila, Auli Kinnunen, Marja-Leena Karjalainen and Seppo Lähdesmäki. Risto Helminen is acknowledged for his technical assistance with the computers.

I want to express my warmest gratitude to my parents, Reijo and Vuokko, for setting me an example and providing a loving family throughout the years. I cannot thank you enough for giving me such an excellent basis for my life and always being there when needed. I also value the friendship and joyful memories with Kaisa, Antti and Satu, my dear siblings. Parents-in-law and colleagues Jaakko and Raija are to thank for their support and precious times spent together. I also want to thank my good friend Pasi and other mates who walked beside me during the long and challenging path to becoming a medical doctor. Mia and
Kimmo, my dear friends, are also to be thanked for their friendship and all their help.

Finally, without the love and encouragement of my beloved wife this work would have never been accomplished. Minna, you have lifted me up and made me a better man. I cherish everything we have experienced while going through medical school together and starting a family. I cannot think of a better mother for our precious little son Kalle. You truly are my eternal companion and best friend.

This work was supported by grants from the Finnish Medical Foundation Duodecim and the Oulu University Foundation.

Oulu, May 2009

Olli-Pekka Kämäräinen
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>articular cartilage</td>
</tr>
<tr>
<td>ADAMTS</td>
<td>a disintegrin and metalloproteinase with thrombospondin motifs</td>
</tr>
<tr>
<td>BMP</td>
<td>bone morphogenetic protein</td>
</tr>
<tr>
<td>CDMP1</td>
<td>cartilage-derived morphogenetic protein 1</td>
</tr>
<tr>
<td>Chr</td>
<td>chromosome</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CILP</td>
<td>cartilage intermediate layer protein</td>
</tr>
<tr>
<td>cM</td>
<td>centimorgan</td>
</tr>
<tr>
<td>CMC</td>
<td>carpometacarpal</td>
</tr>
<tr>
<td>COL</td>
<td>collagenous domain</td>
</tr>
<tr>
<td>COMP</td>
<td>cartilage oligomeric matrix protein</td>
</tr>
<tr>
<td>CS</td>
<td>chondroitin sulfate</td>
</tr>
<tr>
<td>DIP</td>
<td>distal interphalangeal</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>ERT</td>
<td>estrogen replacement therapy</td>
</tr>
<tr>
<td>FACIT</td>
<td>fibril-associated collagen with interruptions in the triple helix</td>
</tr>
<tr>
<td>FGF</td>
<td>fibroblast growth factor</td>
</tr>
<tr>
<td>G</td>
<td>guanine nucleotide base</td>
</tr>
<tr>
<td>GAG</td>
<td>glycosaminoglycan</td>
</tr>
<tr>
<td>GDF-5</td>
<td>growth/differentiation factor 5</td>
</tr>
<tr>
<td>GWA</td>
<td>genome-wide association</td>
</tr>
<tr>
<td>HA</td>
<td>hyaluronan</td>
</tr>
<tr>
<td>HOA</td>
<td>hand osteoarthritis</td>
</tr>
<tr>
<td>hr</td>
<td>human recombinant</td>
</tr>
<tr>
<td>HS</td>
<td>heparan sulfate</td>
</tr>
<tr>
<td>ICD</td>
<td>international classification of diseases</td>
</tr>
<tr>
<td>ICE</td>
<td>IL-1β converting enzyme</td>
</tr>
<tr>
<td>IGD</td>
<td>interglobular domain</td>
</tr>
<tr>
<td>IGF</td>
<td>insulin-like growth factor</td>
</tr>
<tr>
<td>IIHH</td>
<td>Indian hedgehog</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>JSN</td>
<td>joint space narrowing</td>
</tr>
<tr>
<td>KL</td>
<td>Kellgren &amp; Lawrence</td>
</tr>
<tr>
<td>KS</td>
<td>keratan sulfate</td>
</tr>
<tr>
<td>LD</td>
<td>linkage equilibrium</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>LIF</td>
<td>leukemia inhibitory factor</td>
</tr>
<tr>
<td>LOD</td>
<td>logarithm of odds</td>
</tr>
<tr>
<td>LP</td>
<td>link protein</td>
</tr>
<tr>
<td>MED</td>
<td>multiple epiphyseal dysplasia</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloproteinase</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>NC</td>
<td>noncollagenous domain</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>PCM</td>
<td>pericellular matrix</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PIP</td>
<td>proximal interphalangeal</td>
</tr>
<tr>
<td>PSACH</td>
<td>pseudoachondroplasia</td>
</tr>
<tr>
<td>RA</td>
<td>rheumatoid arthritis</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>SLRP</td>
<td>small leucine-rich proteoglycan</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>TACE</td>
<td>TNF-α converting enzyme</td>
</tr>
<tr>
<td>TGF</td>
<td>transforming growth factor</td>
</tr>
<tr>
<td>TIMP</td>
<td>tissue inhibitor of metalloproteinase</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
<tr>
<td>VNTR</td>
<td>variable number of tandem repeats</td>
</tr>
<tr>
<td>VWA</td>
<td>von Willebrand factor A</td>
</tr>
<tr>
<td>Wnt</td>
<td>wingless-related MMTV integration site</td>
</tr>
<tr>
<td>X</td>
<td>any amino acid</td>
</tr>
<tr>
<td>Y</td>
<td>any amino acid</td>
</tr>
</tbody>
</table>
**List of original articles**

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


*These authors contributed equally to this study.
Contents

Abstract
Acknowledgements
Abbreviations
List of original articles
Contents
1 Introduction
2 Review of the literature
  2.1 The structure and formation of the synovial joints
  2.2 Cartilage
    2.2.1 General structure of articular cartilage
    2.2.2 The chondrocyte
    2.2.3 The extracellular matrix (ECM)
  2.3 Osteoarthritis (OA)
    2.3.1 Symptoms and clinical characteristics of osteoarthritis
    2.3.2 Prevalence of osteoarthritis
    2.3.3 Etiopathogenesis of osteoarthritis
    2.3.4 Relevance of inflammation in osteoarthritis
  2.4 Inheritance of osteoarthritis
    2.4.1 Association analyses of candidate genes
    2.4.2 Genome-wide studies
3 Outlines of the present study
4 Materials and methods
  4.1 Study populations
  4.2 Southern hybridization
  4.3 Linkage analysis
5 Results
  5.1 Aggrecan VNTR polymorphism and hand osteoarthritis
  5.1.1 VNTR allele frequency
  5.1.2 The most common aggrecan VNTR allele, A27, is protective against hand osteoarthritis
  5.2 Interleukin 6 promoter polymorphisms and symptomatic distal interphalangeal osteoarthritis
    5.2.1 G-alleles of the promoter polymorphisms associate with symptomatic distal interphalangeal osteoarthritis
    5.2.2 Haplotype analysis of the promoter polymorphisms
5.3 Interleukin 1 gene cluster polymorphisms and bilateral distal interphalangeal osteoarthritis (III) ........................................................... 69
  5.3.1 Frequency of the alleles, genotypes and carriage rates of the studied polymorphisms ........................................................... 70
  5.3.2 Analysis of the extended haplotypes ........................................... 72
5.4 Linkage analysis of primary hip and knee osteoarthritis (IV) .......... 73
  5.4.1 Genome-wide scan .................................................................... 74
  5.4.2 Fine mapping ............................................................................. 74
  5.4.3 Association analyses of potential candidate genes ....................... 75
6 Discussion .................................................. 77
  6.1 The complex genetics of osteoarthritis ........................................... 77
  6.2 Characteristics of the study populations ....................................... 78
  6.3 Is the effect of the aggrecan VNTR polymorphism significant in the development of osteoarthritis? (I) ..................................................... 79
  6.4 Genetic factors affect the inflammation reaction observed in osteoarthritis cartilage (II, III) ............................................................... 81
  6.5 Linkage analysis, a way to identify new osteoarthritis-associated chromosomal loci (IV) ............................................................... 83
7 Conclusions .................................................. 85
References .................................................. 87
Original articles ........................................... 111
1 Introduction

The synovial joint is macroscopically a rather simple structure that connects two bones together, allowing almost frictionless motion between the skeletal surfaces. The development of joints is thought to arise from the mesenchymal cells in a process that is still rather unclear and leads to the formation of the fluid-filled synovial cavity, with articular cartilage (AC) covering the ends of the bones and the surrounding synovial capsule that protects the structure. Joint cartilage is composed of a small amount of chondrocytes that synthesize the rest of the large surrounding extracellular matrix (ECM), consisting mainly of collagen II fibrils and tens of different proteoglycans and other macromolecules. The homeostasis of AC is maintained by chondrocytes, but affected also by synovial cells and osteocytes of the subchondral bone via secretion of different regulatory and signaling molecules. The structure of the collagen-proteoglycan network in AC gives the joint its biomechanical properties by allowing hydration of glycosylated macromolecules to create osmotic pressure within the tissue, empowering it to sustain continuous mechanical pressure.

The most common disease affecting the synovial joints is osteoarthritis (OA). It is a chronic, often slowly developing process that leads to the degeneration and erosion of AC, causing pain and the inability to use the affected joint. OA affects a substantial proportion of the Western populations causing personal suffering as well as substantial financial burden. The breakdown of cartilage in OA is a result of an imbalance between catabolic and anabolic processes within the AC involving the structural molecules as well as various regulatory elements. Despite the fact that many individual pathological processes taking place in OA-affected cartilage have been described, the etiopathogenesis of the disease is still, in many respects, under debate. OA can be caused by obesity, continuous excess of mechanical stress or joint trauma and it affects most often the joints of the hand, hip and knee. OA is more prevalent among women, and the overall prevalence of the disease is highly associated with aging. In a substantial amount of OA cases, no specific etiologic factor can be identified. However, the disease clearly accumulates in certain families and has been shown to involve a significant heritable component. There is currently no cure available for this condition and the process of joint degeneration cannot be stopped once it has begun. Treatment is mostly based on relieving the pain symptoms and ultimately on joint replacement therapy.
Genes are believed to be the answer to the unknown etiology of OA. To date, dozens of genetic variations across the genome have been reported to associate with the disease. Yet, the results have been modest and no major disease-causing factor has been identified. Association analyses have not supported the idea that the genes of the main structural proteins of AC are major players in OA. More convincing associations have been reported concerning the genes of different regulatory and signaling molecules present in cartilage. Besides association analyses of the susceptibility genes, information about the genetics of OA has been sought from genome-wide linkage analyses of disease-affected families. This strategy has resulted in the identification of many chromosomal regions that are likely to harbor previously unknown OA predisposing variations. Overall, the genetics of OA have proven to be complex and the contribution of independent genetic factors may differ depending on the ethnicity and phenotype of the disease.

In this study, sequence variations in the genes of one major structural protein of AC and several significant inflammatory mediators were analyzed in order to evaluate their possible contribution to the development of OA of the hand. Also, a genome-wide linkage analysis was performed using families with multiple members affected with hip and knee OA. The purpose was to identify new chromosomal loci that are likely to contain previously unidentified disease-associated variants.
2 Review of the literature

2.1 The structure and formation of the synovial joints

Synovial joints are essential for organism function, movement and quality of life. Early investigators identified four different types of joints. The synovial joint was described as eudiarthrosis, having separate articulating elements with a joint cavity limited by synovial tissues. The other three types of joints included synarthrosis, where cartilage or other connective tissue binds skeletal elements together, schizarthrosis, where the space between the skeletal elements consists of connective tissue and a number of usually small cavities, and periarthrosis, where skeletal elements unite around a single joint cavity (Haines 1941, 1942). Joints can also be classified in terms of the movement which they allow. There is no movement, for example, between cranial bones because they are connected by synarthrosis. A limited movement is possible between the vertebrae provided by the flattened discs of fibrocartilage (amphiarthroses). Only the synovial joints can permit a wide range of movement. They are also referred to as diarthroses in the current literature. The function of a synovial joint is to allow smooth articulation between opposing skeletal elements. Biomechanical loads are transmitted through this structure comprised of articular cartilage (AC) that covers the ends of the bones, synovial fluid that lubricates the cartilaginous surfaces and nourishes the avascular tissue, ligaments that bind the skeletal elements together, and a fibrous capsule that protectively encapsulates the joint (Figure 1). The synovial joint may contain meniscal structures internally. The AC is a fibrous structure and is continuous with the periosteum. It is important in stabilizing the joint. The capsule is lined by the synovial membrane, which secretes the synovial fluid required for low-friction articulation. Covering the epiphyses of the bones, there is mostly cartilage, which is responsible for maintaining the functional ability of the joint and providing a durable, load-bearing surface against extensive biomechanical strain. (For a review, see Khan et al. 2007.)

The formation of joints is a complex and highly organized process in which the synovial joint cavities must eventually generate two opposing, non-adherent surfaces facilitating painless and almost frictionless articulation. This process also involves the creation of a cell-free, fluid-filled separation (Lamb et al. 2003). The main phases of joint development are presented schematically in Figure 1 and described in more detail below.
According to current understanding, the formation of joints begins with the migration of undifferentiated mesenchymal cells to areas destined to become bone and joints. First, in the developing embryo, the condensation of mesenchymal prechondrogenic cells in the early limb manifests no signs of joint formation. The first recognized event in joint formation is the condensation of mesenchymal cells to form the so-called interzone at the future location of the joint (Holder 1977, Mitrovic 1978). The interzone varies between species, being a thicker three-layered structure in chick bone elements (Craig et al. 1987), but rather thin in developing mammalian joints. In humans this cell layer is flattened, consisting of two to three cells (Edwards et al. 1994). The interzone can be ultrastructurally distinguished as two outer layers that are contiguous to the epiphyseal ends and a thin central intermediate zone. Articular cartilage that covers the epiphyses of the opposing bones has been thought to derive from the cells of the intermediate layer (Archer et al. 1994, Ito & Kida 2000, Pacifici et al. 2006).

Following the interzone formation and chondrogenic differentiation, the joint cavity begins to form between two cartilaginous elements that are growing against each other through forces largely generated by hypertrophy and ECM secretion within the elements. This force is generated through the upregulation of hyaluronan (HA) in the cells of the interzone, articular surface and synovium and is dependent on the movement of the embryo (Khan et al. 2007). At present, it is a widely accepted theory that the accumulation of HA contributes to the loss of tissue integrity, causing the separation of the opposing joint sides and formation of the fluid-filled cavity (Pacifici et al. 2005). Another, older theory of joint cavitation is based on selective cell degeneration and increased apoptosis within the interzone (Mitrovic 1978). After the recognition of necrotic features in the cells of the middle interzone, this theory has been widely studied. Despite several more recent reports of observed cell death in the intermediate zone of the interzone in avian and mammalian embryos (Nalin et al. 1995, Kimura & Shiota 1996, Abu-Hijleh et al. 1997), it is still under debate whether or not cell death is an important contributor to joint formation or if the local changes in the ECM and mechanical factors are independently responsible for cavitation. Details of the three-dimensional configuration, shape and organization of the joints are the least understood aspects of joint formation, likewise is the morphogenesis of the whole embryo. (For reviews, see Lamb et al. 2003, Pacifici et al. 2005 and Khan et al. 2007.)
The interzone expresses a wide variety of regulatory and signaling molecules. Wingless-related MMTV integration site (Wnt) 14, a secreted growth factor of the Wnt-gene family, is highly expressed in joint-forming regions in the interzone and is essential in initiating synovial joint formation (Hartmann & Tabin 2001). Localized production of Wnt-14 induces the expression of growth/differentiation factor 5 (GDF-5), a mouse homolog of cartilage-derived morphogenetic protein 1 (CDMP1). It is expressed in the developing joints throughout the cavitation process and is believed to promote interzone cell function and joint development (Storm & Kingsley 1999, Shum & Nuckolls 2002). Also, bone morphogenetic protein (BMP) antagonists are considered to be important in the regulation of chondrogenesis and joint formation (Pacifici et al. 2005). In addition, Indian hedgehog (IHH), a signaling factor produced by maturing chondrocytes, is a critical and possibly direct regulator of joint development (Koyama et al. 2007).

In summary, many of the molecules that contribute to joint formation have been identified and the main phases in of the process described; however the
detailed biology of joint formation and morphogenesis remains to be uncovered by future studies. (For more detailed reviews, see Lamb et al. 2003, Pacifici et al. 2005 and Khan et al. 2007.)

2.2 Cartilage

Three different types of specialized connective tissue, known as cartilage, exist in the human body: hyaline cartilage, elastic cartilage and fibrocartilage. Hyaline cartilage forms the cartilaginous model of the developing skeleton. It is replaced by bone in a process known as endochondral ossification. In adults, hyaline cartilage exists in the AC of joints and the cartilage of the respiratory tract (nasal, laryngeal, tracheobronchial and costal cartilage). Hyaline cartilage is composed of a small amount of chondrocytes that synthesize the rest of the large surrounding ECM, consisting mainly of collagen II fibrils and proteoglycans. (The structure of ECM and cartilaginous collagens are later reviewed in more detail.) Elastic cartilage is distinguished from hyaline cartilage by the elastic fibers it contains in the ECM. Due to elastin secreted by chondrocytes, elastic cartilage has a remarkable ability to regain its original shape after deformation. Elastic cartilage can be found in the pinna of the ear, epiglottis and in the arytenoid cartilage of the larynx. Fibrocartilage consists of chondrocytes and fibroblasts surrounded by type I collagen. It provides great tensile strength and is found in the intervertebral disks, tendinous and ligamentous insertions, menisci and the symphysis pubis. Characteristic to all these cartilage types is the lack of blood vessels, low amount of cells and a substantial ECM. (Kierszenbaum 2007.)

2.2.1 General structure of articular cartilage

The structure of articular cartilage has fascinated scientists for centuries. Considering the contemporary technology of the time, the structure of AC was described amazingly adequately as early as the mid-eighteenth century, by the famous London anatomist and surgeon William Hunter (1743). He, as well as other former scientists, had been intrigued especially by the fine composition, nourishment and stability of AC, which holds such unique biomechanical properties (Benedek 2005). Today, the general structure of this tissue is known to consist of four different horizontal layers that vary in size among different joints. The thinnest of the layers is the superficial zone beginning from the articular surface bathed by synovial fluid. Here, the density of chondrocytes is highest, and
cells are flattened and situated parallel to the articular surface. Densely packed bundles of collagen II fibrils run between the chondrocytes. Collagen fibrils are also parallel close to the surface, contributing to the mechanical properties. Deeper in the superficial zone, collagen fibrils run vertically. The second layer is the midzone, where cells are more rounded and situated more broadly. The ECM is more extensive in the midzone and collagen fibrils are arranged randomly. Cell volume is at its lowest in the deep zone, where collagen content is minimal, but the fibril diameter is maximal. Situated between the deep zone and underlying subchondral bone is the calcified zone. The deep zone is separated from the calcified zone by a wavy, irregular line known as the tide mark. The outline of the tide mark is highly variable. Structurally, it may prevent collagen fibrils in the noncalcified zone from anchoring to the calcified zone. The calcified zone provides a buffer with intermediate mechanical properties between those of the uncalcified cartilage and the subchondral bone. Here, the chondrocytes are located in uncalcified lacunae. The collagen fibrils, large in diameter, are arranged perpendicular to the articular surface and are anchored in a calcified matrix. From the calcified layer, the dynamic forces are transmitted to the underlying subchondral bone. A schematic drawing of the orientation of collagen fibrils and chondrocytes in AC is presented in Figure 2. (For reviews, see Huber et al. 2000 and Poole et al. 2001.)
Fig. 2. Schematic drawing of collagen fibrils and chondrocytes in cartilage. In the superficial zone, the collagen fibrils run parallel to the articular surface and lie close to each other in a dense arrangement. The collagen fibrils in the middle zone are randomly oriented and are more loosely packed. In the deep zone, the collagen fibrils orient themselves perpendicular to the subchondral bone surface. Percentages describe the estimated volume of each zone of the total cartilage volume. (From Ge et al. 2006. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

The dry weight of adult AC is two-thirds collagen (Eyre et al. 2006). Another major structural component of AC is a noncollagenous large aggregating proteoglycan, aggrecan (van der Kraan et al. 2002). Collagen II is the major collagen and is crosslinked together with minor collagens IX and XI, thus forming the framework of the ECM (van der Rest & Mayne 1987). The collagen construct embedded with glycoprotein aggregates gives the tissue its form and enables tensile strength and the ability to endure compression.

2.2.2 The chondrocyte

Arising from the mesenchymal stem cells, the chondrocyte is a unique cell type in AC tissue. In adult humans, chondrocytes account for approximately 1–5% of the AC tissue volume. Chondrocytes are non-uniform and their number and shape vary in different zones of the AC as described above. Chondrocytes are the only cell type found in the AC and are responsible for production of the surrounding network of ECM proteins and their organization into a functional structure.
Chondrocytes control the turnover of the ECM proteins and their metabolic activity varies between the different layers of AC (Huber et al. 2000). There are no blood vessels in AC, thus the metabolism of chondrocytes is essentially anaerobic as the nutrients are received by diffusion from the synovial fluid and, in a very small measure, from the subchondral bone (Corvol 2000). Chondrocyte metabolism operates at low oxygen levels that range from 1% (deep zones) to 10% at the surface. Nevertheless, chondrocytes can respond to mechanical stimuli, growth factors and cytokines that influence normal homeostasis (Goldring 2006). Glucose is the major energy source for these cells and an essential precursor for matrix glycosaminoglycan (GAG) synthesis, whose importance for the biomechanical properties of AC will be reviewed later. In adult joint cartilage, chondrocytes undergo a slow maturation that slows with age. At the same time, the turnover rate of matrix proteins decreases gradually, slowly weakening the AC. (Corvol 2000.)

2.2.3 The extracellular matrix (ECM)

The structure that enables the joint to endure an extreme amount of pressure (up to ten times the body weight) and continuous mechanical stress is the solid organic matrix, commonly referred to as the ECM. It consists of three principal phases: (i) a solid phase composed mainly of a densely woven collagen fibrillar network enmeshed with a high concentration of charged proteoglycan aggregates, (ii) a fluid phase of mainly water, gases, small proteins and metabolites, and (iii) an ion phase with many ionic species of dissolved electrolytes required to neutralize the charges fixed to the solid matrix (Mow et al. 1999). From the cell outwards, the ECM is divided into the pericellular, territorial and interterritorial regions (Figure 3), each region having its own structure and specific distribution of proteoglycans (Poole et al. 1982, Aigner & Stöve 2003).

Chondrocytes are surrounded singly or in groups by a narrow region called the pericellular matrix (PCM), which together with the cell has been termed the chondron. It is a 3-μm-thick pericellular microenvironment rich in collagen types II, IX, XI and especially type VI, which distinguishes the area from the ECM. The PCM is also rich in hyaluronan, glycoproteins such as fibronectin, and different proteoglycans. (Ross et al. 2006.) It is believed that the biochemical and biophysical signals received by chondrocytes are influenced by the surrounding PCM, which may also play an important role in the response of cells to mechanical compression (Guilak et al. 2006). In addition, the PCM may serve as
a protective layer for the chondrocytes (Choi et al. 2007). A number of experimental and theoretical studies indicate that the PCM is important in regulating the microenvironment of the chondrocyte; still, further research is needed to improve our understanding of its structure and function in cartilage physiology. (For a more detailed review, see Guilak et al. 2006.)

The interterritorial matrix compartment represents the major portion of the cartilage matrix far from the chondrocytes and consists of the collagen II/IX/XI fibril, aggrecan and various other proteoglycans (Aigner & Stöve 2003). When cartilage ECM is discussed in the literature, it usually refers to this interterritorial matrix. The territorial matrix lies between the interterritorial and pericellular matrix and is under the metabolic control of the chondrocyte (Ross et al. 2006). So far, the cell-associated territorial matrix region lacks a specific biochemical characterization (Aigner & Stöve 2003). The properties of different collagen types, proteoglycans and other significant matrix molecules as well as their known functions in the AC are described in more detail in the following sections. The implications of the varying genetic characteristics of these molecules in OA are reviewed later, in section 2.4.
Fig. 3. Electron microscopic image of the ECM compartments. The extracellular matrix of the AC can be subdivided into the pericellular, territorial and interterritorial compartments (a); at the ultrastructural level, the pericellular matrix appears as fine filamentous material (partly type VI collagen) (c,d); the interterritorial cartilage matrix shows the typical cross-striated type II collagen fibrils in a network-like arrangement and the amorphous non-fibrillar matrix in between (b). (Adapted from Aigner & Stöve 2003, with permission from Elsevier.)

Collagenous components of the ECM

The superfamily of collagens. At present, 29 different triple-helical ECM proteins have been named as collagens. These proteins form a superfamily that constitutes about 30% of the total protein mass in the human body. Collagens have a plethora of essential biological functions including maintaining of the structure of various tissues, such as AC. A structure consisting of three polypeptide chains, called α chains, is distinctive to all collagens. Each α chain contains a repeating Gly-X-Y sequence (X and Y meaning any of the 20 amino acids in proteins), where proline is often found in the X position. Forty-two different polypeptide chains are known
to date, and the three \( \alpha \) chains in the mature protein can be identical or vary depending on the type of collagen. Being in every third position, the smallest amino acid, glycine, enables the coiling of the \( \alpha \) chains into a triple-helical structure, important for the functioning of these molecules. Posttranslational modification by specific enzymes is typical for collagens. This often includes the hydroxylation of proline in the Y position, which is important for the stability of the triple helix.

In the ECM, most collagens form supramolecular assemblies and can be divided into several subfamilies according to the three-dimensional construction of the aggregate. In cartilage tissue, a major structural role is played by fibril-forming collagens (collagens II and XI) and fibril-associated collagen with interruptions in the triple helix (FACTTs) (collagen IX). The tissue distribution of collagen varies substantially, as the foregoing collagen types are found almost exclusively in cartilage, whereas e.g. collagen VI, which forms beaded filaments, occurs in most connective tissues including cartilage. (For reviews, see Myllyharju & Kivirikko 2001, Myllyharju & Kivirikko 2004 and Heino 2007.)

Collagens II, IX and XI are referred to as cartilage-specific collagens, and together they form a copolymer that is indispensable to the structure of cartilage. Collagen II represents over 90%, collagen IX about 1% and collagen XI about 3% of the total collagen in the cartilage matrix (Eyre et al. 2006). Mutations in any of the genes coding for these collagens have been observed to cause diseases affecting connective tissue that vary from barely detectable mild conditions to lethal (for reviews, see Myllyharju & Kivirikko 2001 and 2004).

**Collagen II.** The fibril-forming collagen II is the foundation of the hyaline cartilage, representing about 80% of the tissue’s collagen content. It is a homotrimer consisting of three identical \( \alpha_1 \) (II) chains coiled into triple-helical fibrils, which are covalently crosslinked together to form a polymer of axially staggered periodic fibrils, providing collagen its tensile strength (Eyre 1991). The crosslinked polymers further assemble into supramolecular bundles forming a framework for the ECM. The deployment of the fibrillar network varies between the different layers of AC, as discussed previously. Collagen II is also found in the vitreous humor of the eye, in the nucleus pulposus and annulus fibrosus of the intervertebral disks, in the inner ear and in some non-chondrogenic tissues during development (Myllyharju & Kivirikko 2001, Gelse et al. 2003). The protein is coded by a single gene, **COL2A1**, which is located on chromosome 12q13.11-13.12 and consists of 54 exons (Ala-Kokko & Prockop 1990, Takahashi et al. 1990, Ala-Kokko et al. 1995). Collagen II, as well as other fibril-forming
collagens, is synthesized in precursor form. Alternative splicing of exon 2 results in the generation of two different forms of procollagen II (IIA and IIB), of which IIB is primarily expressed by chondrocytes (Sandell et al. 1994). Following or during the secretion of procollagen into the ECM, N- and C-terminal propeptide extensions are cleaved by specific procollagen proteinases, thereby triggering the fibril formation (Hulmes 2002). The association of the collagen II bundle with other cartilage collagens and the noncollagenous components of cartilage are presented in Figure 4.

**Collagen IX.** Collagen IX belongs to the family of FACIT collagens, which are characterized by the presence of two highly conserved cysteine residues separated by four amino acids at the NC1-COL1 junctions, the existence of two Gly-X-Y triplet imperfections within the COL2 domain, a succession of triple-helical domains connected by short noncollagenous domains (NC), and the presence of a large N-terminal domain containing a thrombospondin subdomain next to the collagenous region (Ricard-Blum & Ruggiero 2005). FACIT collagens do not form fibrils, but associate with fibrillar collagens. Collagen IX is composed of three genetically different α chains, α1(IX), α2(IX) and α3(IX), encoded by the COL9A1, COL9A2 and COL9A3 genes, which are all mapped to different chromosomes. Each chain forms three triple-helical collagenous domains, COL1-3, flanked by noncollagenous domains NC1-4, and the mature polypeptide forms a rod-like structure (Olsen 1997). This collagen type has also been considered to be a proteoglycan, since it carries an attachment site for GAGs in the COL3 domain of the α2(IX) chain (McCormick et al. 1987). Still, no distinct function for the proteoglycan feature has been demonstrated. Collagen IX has at least seven cross-linking sites, and in cartilage it is covalently bound to the collagen II decorating the surface of the fibril, with a space between the individual molecules. It is also able to covalently bind to other collagen IX molecules. (Eyre et al. 2006.) Collagen IX has shorter and longer variants due to the use of two distinct promoters in the α1(IX) collagen gene (Nishimura et al. 1989). The longer collagen IX variant is the major form in cartilage. It contains a large NC4 domain at the tip of the COL3 domain, which projects out of the fibril surface and may allow possible interaction of the NC4 domain with matrix proteoglycans and other molecules. The shorter collagen IX, lacking the NC4 domain, is found in the vitreous of the eye, intervertebral disk (IVD) and various developing non-chondrogenic tissues (Olsen 1997, Wu & Eyre 2003).

Collagen IX is an essential part of the collagen II/IX/XI heteropolymer in AC as it gives stability to the collagenous framework and is suggested to be a
macromolecular bridge between fibrils and other matrix constituents. It is most abundant in the areas of thinner fibrils that form the chondron and may also have a role in regulating the diameter of the collagen fibrils. (For reviews, see Eyre & Wu 1995, Olsen 1997, Hagg et al. 1998, Eyre et al. 2002, Gelse et al. 2003 and Eyre et al. 2006.)

**Collagen XI.** The quantitatively minor fibril-forming collagen XI is the third important component of the AC collagen network, where it is buried within the major collagen II fibril. It is a heterotrimeric molecule composed of three α chains, α1(XI), α2(XI) and α3(XI), that are products of three distinct genes, *COL11A1, COL11A2* and *COL2A1*, all located in different chromosomes. The α3(XI) is a product of the same gene that encodes collagen II, but is more extensively modified posttranslationally (Wu & Eyre 1995). Collagens II and XI are structurally related, differing mainly in their N-propeptides. Collagen XI contains a long triple-helical part (COL1), short N-terminal triple-helical part (COL2) and three noncollagenous domains (NC1-3) in each α chain. In contrast to other fibrillar collagens, α1(XI) chains retain at least to some extent their N-terminal propeptides even as mature molecules (Wu & Eyre 1995). These retained propeptides are thought to regulate the fibrillogenesis of collagen II (Gregory et al. 2000). Collagen XI is closely related to collagen V in biological and structural features, suggesting that these two collagens constitute a single type of collagen form, V/XI collagen (Fichard et al. 1995). Crosslinked to each other in a head-to-tail manner, collagen XI molecules are likely to form a template that constrains the lateral growth of the collagen II fibrils as well as stabilize them (Blaschke et al. 2000). Even though collagen XI represents a relatively small amount of the collagen in cartilage fibrils, its presence is essential for the regulated assembly, organization and development of cartilage (Gregory et al. 2000).
**Fig. 4.** Schematic presentation of cartilage ECM showing a heterotypic collagen fibril consisting of collagens II, IX and XI, and its association with some of the noncollagenous components of cartilage, including aggrecan, cartilage oligometric matrix protein (COMP), hyaluronan, matrilin-3 and link protein. The figure has been originally published in Eveliina Jakkula’s thesis, Acta Universitatis Ouluensis, D832.

Other collagens in cartilage. Other collagens are also present in cartilage, but are not considered cartilage-specific due to their main expression in non-cartilaginous tissues. Collagen III, for example, is quite abundantly present in cartilage matrix accounting for some 10% of the collagen mass (Eyre et al. 2006). It has been found to associate with collagen II in the same banded fibrils (Young et al. 2000), perhaps as part of the matrix repair or remodelling processes (Eyre et al. 2006). Collagen VI is a beaded filament that forms dimers and tetramers. It is a heteropolymer of three distinct α chains that are bundled together containing triple-helical domains as well as N- and C-terminal domains. In normal articular cartilage, it is exclusively present in the PCM, where it interacts with different ECM components including collagen II, and may also form a network that anchors the chondrocyte to the PCM. (Guilak et al. 2006.) Collagen X is expressed only by hypertrophic chondrocytes and is shown to form latticework in the AC (Bruckner & van der Rest 1994). Articular cartilage also contains a small amount of collagens XII, XIII, XIV and XVI (Kassner et al. 2003, Eyre et al. 2006). In addition, collagens XX and XXVII have been detected in some cartilaginous tissues (Koch et al. 2001, Pace et al. 2003). So far, relatively little is known about the role of these minor collagens in the functioning of AC, their organization within the collagen II meshwork as well as their influence on development, aging, and disease processes (Young et al. 2000).
Proteoglycans of the ECM

Aggrecan. Proteoglycans constitute a family of glycoconjugates with a central core protein to which GAG side chains are covalently attached (Wight et al. 1992). Cartilage contains up to 10% of proteoglycans consisting primarily of aggrecan (90% of the total cartilage proteoglycan mass) (Kiani et al. 2002). Carrying a substantial amount of GAG chains of keratan sulfate (KS) and chondroitin sulfate (CS) on its protein core, the aggrecan molecule greatly resembles a bottle brush in shape. It interacts with HA and link protein (LP) to form large aggregates, each consisting of up to 100 aggrecan molecules radiating from the central HA string (Roughley 2006a). The multiple GAG side chains attached to aggrecan include negatively charged anionic groups resulting in an imbalance in ion concentration between the cartilage and surrounding tissue.

The osmotic imbalance causes a large amount of water (about 70% by wet weight of the tissue) to be drawn into cartilage causing swelling and expanding of matrix. Large swollen glycoprotein aggregates are entrapped within the organized collagen network, forming a space-filling gel critical to the biomechanical properties of cartilage. (Kiani et al. 2002, Dudhia 2005.) In this manner, aggrecan provides the dominant mechanism of energy dissipation in shock absorbing tissues and endows AC with strength against compression and mechanical strain (Rizkalla et al. 1992, Iozzo 1998, Papagiannopoulos 2006). The concentration of aggrecan is lowest in the ECM near the articular surface, where tensile strength is mainly provided by tight collagen bundles, and increases when moving into the deeper zones of AC (Poole 2001). A schematic presentation of the structure of aggrecan and its role in forming a large proteoglycan aggregate is presented in Figure 5.
The human aggrecan gene, *AGC1*, is located on chromosome 15q26 (Korenberg *et al.* 1993). It consists of 19 exons with an organization that strongly correlates to the specific domains of the core protein (Valhmu *et al.* 1995). The core protein of aggrecan consists of three disulphide-bonded globular domains (G1, G2 and G3). G1 and G2 domains are connected by an extended interglobular domain (IGD), and a large sequence characterized by KS and CS side chains is situated between the G2 and G3 domains (Kiani *et al.* 2002).

Beginning from the N-terminal region, the G1 domain encoded by exons 3–6 (Roughley 2006a) is involved in the interaction with HA and LP, gluing all three together thus stabilizing the matrix network. The G1 domain also mediates interactions between chondrocytes and the matrix network, regulates product processing, and maintains proteoglycan quality control (Kiani *et al.* 2002). The IGD, encoded by exon 7 of *AGC1*, contains proteolytic cleavage sites for different proteinases such as matrix metalloproteinases (MMPs), serine proteases and acid proteases (Hardingham & Fosang 1995, Mort *et al.* 1998). Among these proteinases is aggrecanase-1, a member of the protein family of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), which is able to cleave aggrecan at a specific site in the IGD region (Tortorella *et al.* 1999).
addition, cytokines target the degradation of aggrecan via cleavage at the proteolytic sites in the IGD region (Loulakis et al. 1992). Aggrecan cleavage in the IGD region results in rapid loss of the whole GAG region; thus, IGD appears to be involved in the physiological turnover of aggrecan (Kiani 2002). The G2 domain is encoded by exons 8–10 (Roughley 2006a). Its exact function is still unknown, but it seems that this domain inhibits aggrecan secretion and ensures that only fully glycosylated aggrecan monomers are secreted, thereby maintaining product quality control (Kiani et al. 2001). The CS-rich domain and much of the KS-rich domains are encoded by the large exon 12 (Roughley 2006a) and constitute the most dominant feature of aggrecan, making up some 80% of the protein’s molecular mass (Dudhia 2005). About 30 KS and 100 CS chains are attached to the protein core and contribute approximately 8000–10000 negatively charged groups to the molecule, resulting in the osmotic activity described above (Dudhia 2005). Interestingly, and unique in humans, a variable number of tandem repeat (VNTR) polymorphism in the aggrecan gene area encoding the CS domain results in a range of core protein sizes and the ability to bear different numbers of CS chains depending on the length of the tandem repeat sequence (Doege et al. 1997).

Lastly, in the C-terminal region is the G3 domain encoded by exons 13–18 (Roughley 2006a). It is a complex region produced by alternative splicing of exons during posttranslational processing and appears to be involved in the processing of the proteoglycan, while also facilitating GAG chain attachment and enhancing product secretion (Kiani et al. 2002). The G3 domain is known to be removed from aggrecan in mature cartilage, probably by proteolytic cleavage, and it also seems to decrease with aging (Dudhia et al. 1996). (For reviews, see Kiani et al. 2002, Dudhia 2005 and Roughley 2006a.)

Other proteoglycans of the extracellular matrix. Of the family of aggregating proteoglycans, versican is also expressed in cartilage, but at much lower levels than aggrecan (Roughley 2001). The large proteoglycan aggregates are stabilized by a small proteoglycan LP, which is also needed in the process of aggregate formation (Melching & Roughley 1990). In addition, LP forms a protein coat covering the surface of HA and protecting it from degradation (Roughley 2006a). HA binds to the collagen framework and is essential in the retention of aggrecan in the ECM (Poole et al. 1982). The fundamental role of both LP and HA in normal cartilage function has been demonstrated in mouse models (Watanabe & Yamada 1999, Roughley 2006a). (For reviews, see Huber et al. 2000 and Roughley 2006a.)
The ECM also contains different leucine-rich repeat proteoglycans (SLRPs) known as decorin, biglycan, fibromodulin and lumican, which are minor components in size. All of them consist of a core protein and a different amount of attachment sites for GAGs of either CS or KS (Hocking et al. 1998, Iozzo 1999). SLRPs are most concentrated in the surface layer or AC, where they bind to the other matrix macromolecules, especially to collagen fibrils, helping to regulate fibril diameter during its formation and possibly fibril-fibril interactions as well as giving stability to the matrix (Huber et al. 2000, Geng 2006, Roughley 2006a). SLRPs are also able to interact with different growth factors, thus affecting chondrocyte metabolism (Hildebrand et al. 1994, Schönherr et al. 2005). In addition, by providing a coating on the fibril surface, SLRPs seem to be able to protect cartilage collagens from proteolytic degradation by collagenases, also known as the MMPs (Geng et al. 2006). Proline/arginine-rich end leucine-rich repeat protein (PRELP) and chondroadherin also belong to the SLRP family and are found in cartilage ECM (Roughley 2001).

Perlecan, another large cartilage matrix proteoglycan that can bear heparan sulfate (HS) and CS side chains, was originally described as a basement membrane proteoglycan (Iozzo 1998). Perlecan has proved to be an important component in the pericellular matrix and is likely to coordinate proper cartilage growth and development (Gomes et al. 2002). It can bind to growth factors, such as fibroblast growth factor-2, and seems to also have a role in a process where mechanical signals are transduced through cartilage to change chondrocyte gene expression (Vincent et al. 2007).

In addition, there is yet another important glycoprotein operating solely on the surface of AC, that has earlier been poorly understood (Chang et al. 2008). This is a mucinous glycoprotein known as lubricin (also described as superficial zone protein), secreted by surface chondrocytes and synovial cells (Jay et al. 2007). It is indispensable in maintaining frictionless articular movement as it functions as a boundary lubricant between opposing cartilage surfaces (Jones et al. 2007). Lubricin interacts with HA and is able to protect the articular surface, provide synovial fluid with the ability to dissipate strain, and inhibit overgrowth of synovial cells (Rhee et al. 2005, Chang et al. 2008).
Other components of the ECM

Among the structural proteins of ECM, cartilage oligomeric matrix protein (COMP) is suggested to have an important role in matrix assembly. As the fifth member of the thrombospondin gene family, it is a large pentameric glycoprotein expressed at high levels in the interterritorial region of the matrix (Newton et al. 1994, Shen et al. 1995). The function of COMP is still poorly understood. The absence of this protein does not affect normal growth or development (Svensson et al. 2002), but mutations in its gene cause two skeletal dysplasias, pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED) (Briggs et al. 1995, Hecht et al. 1995, Briggs et al. 1998). COMP is likely to mediate the interactions of collagen fibrils with other matrix constituents (Chen et al. 2008). It binds to both collagens II and IX as well as to all of the matrilins and aggrecan (Mann et al. 2004, Pihlajamaa et al. 2004, Chen et al. 2007) and has proved to be important in the fibril formation of cartilage collagens (Halász et al. 2007). (For a review, see also Hecht et al. 2005.)

The matrilins are noncollagenous proteins made up of von Willebrand factor A (VWA) domains, epidermal growth factor-like domains and a coiled coil α-helical module (Deák et al. 1999). Matrilin-1 and -3 are expressed mainly in cartilage and are able to form protein complexes with other matrix components, which is a typical feature for molecules with VWA domains (Wagener et al. 2005). Matrilins are able to interact with at least aggrecan (Hauser et al. 1996), COMP (Mann et al. 2004), some SLRPs (Wiberg et al. 2003), fibrillar collagens (Winterbottom et al. 1992) and collagen VI (Wiberg et al. 2003), serving as adaptors in the assembly of these proteins into supramolecular structures and as modulators of collagen fibrillogenesis (Wagener et al. 2005, Nicolae et al. 2007). The importance of matrilin-1 and -3 has recently been demonstrated by their absence, which causes ultrastructural abnormalities to the AC (Nicolae et al. 2007).

Among the structural components of the ECM, there is also a monomeric glycoprotein that is expressed mainly in the intermediate zone of human cartilage. Due to this localization, it is known as the cartilage intermediate layer protein (CILP) (Lorenzo et al. 1998). Its expression is influenced by TGF-β signaling and the expression substantially increases in association with aging in human articular cartilage; still, the definite function of the protein is unclear (Lorenzo et al. 1998, Mori et al. 2006). CILP seems to be an important component for cartilage
homeostasis and has been linked to various diseases affecting cartilage (Mori et al. 2006).

Moreover, cartilage ECM is comprised of dozens of other proteins that vary greatly in structure, distribution and function. Among the structural components worth mentioning are also fibronectin, fibrillin, C-type lectin, elastin, and tenascin-C. In addition, the matrix harbors different regulatory molecules, such as pleiotrophin, chondromodulins, various growth factors as well as proteinases and inhibitors. (Roughley 2001.)

2.3 Osteoarthritis (OA)

Osteoarthritis is the most common joint affecting disease and the leading cause of disability, particularly among the aging population (Issa & Sharma 2006). OA is characterized by the degeneration and breakdown of articular cartilage, leading to the development of typical symptoms, i.e. pain and stiffness in the affected joint. The onset of OA is strongly related to aging. Other known risk factors include trauma, obesity, over-usage of joints, gender and ethnicity. In addition, the disease also has a strong genetic component. (Haq et al. 2003.) So far, there is no cure for OA as we are unable to repair or stop the degradation of AC once it has started. Therefore, as the disease progresses, the growing rate of disability ultimately leads to the need for joint replacement surgery. Other preceding therapies are mainly focused on treating the symptoms of OA, while the primary cause of this most prevalent joint disease in our society still remains unknown. A review of OA as a disease, its epidemiology, and current understanding of the processes that lead to cartilage destruction in its pathogenesis are presented in the following sections.

2.3.1 Symptoms and clinical characteristics of osteoarthritis

According to the current understanding, OA is not only a disease of AC but involves the entire joint organ, including subchondral bone, menisci if present, ligaments, periarticular muscle, joint capsule and synovium (Hunter & Felson 2006). OA can appear in any synovial joint, but most commonly it affects the joints of the hands, knees, hips and spine (Goldring & Goldring 2006). At the onset of OA, the joint space filled with articular cartilage begins to narrow. Later, shallow fibrillation occurs on the surface of AC, which turns into deeper fissures as the disease progresses. Ultimately, this onward process results in the regional
lack of cartilage called erosions. (Konttinen et al. 2003.) The developing process of the degenerative changes takes a variable amount of time depending on the individual. A typical symptom arising from the developing OA is joint pain, which is usually exacerbated by activity of the joint and relieved by rest. In later stages of the disease, joint pain is often experienced also at rest and at night. (Hunter & Felson 2006.) Characteristic symptoms for OA include reduced joint function and short-term morning stiffness of the affected joint (Konttinen et al. 2003).

When examining a patient suffering from OA, typical findings include reduced function of the affected joint, crepitation, swelling, thickenings of bone around the joint, and tenderness. The diagnosis of OA is usually based on the radiographic imaging of a joint manifesting some or all of the preceding symptoms and findings. In a radiograph of an OA-affected joint, the breakdown of AC is seen as joint space narrowing (JSN). In the more advanced disease, the JSN is accompanied by hypertrophic bone changes, which are seen in radiographic images as a formation of shelves of new bone at the joint margins (osteoophytes), thickening and sclerosis of the subchondral bone, and as the development of subchondral bone cysts. Sometimes in advanced disease, subluxation, or dislocation of the joint, is also seen. In very severe OA, images may show the deformation of the whole joint structure. (For reviews, see Konttinen et al. 2003, Martel-Pelletier 2004, Goldring & Goldring 2006 and Hunter & Felson 2006.)

Classification and grading of the radiographic features of OA have traditionally been based on a generic method described by Kellgren and Lawrence (KL) (1957). In this classification, OA is divided into five grades (0–4) based on the severity of the radiologic findings described above; grade 0 representing definite absence of OA specific changes and grade 4 indicating severe OA (Kellgren & Lawrence 1957). Although the KL scale is widely adopted, it has been criticized for placing too much emphasis on the presence of osteophytes and for causing inconsistencies in the interpretation of the grading descriptors (Menz et al. 2007). Therefore, grading of OA for research purposes is often based on different modifications of the KL scale, and alternative grading systems have also been developed for OA of the hand (Kallman et al. 1989), hip (Croft et al. 1990) and knee (Ahlbäck 1968).

OA is usually classified as primary or secondary. In primary OA, no distinct predisposing environmental factor is identified and the etiology is for the most part unclear. It has a complex genetic component (see the review in more detail in
section 2.4). Most OA patients suffer from primary OA, which can be localized or generalized (affecting multiple joint areas), the latter being more common in postmenopausal women (Haq et al. 2003). Secondary OA has an underlying cause, most commonly trauma, heavy work, obesity or a certain cartilage affecting disorder, such as Paget's disease, rheumatoid arthritis (RA) or other inflammatory arthritis (Haq et al. 2003).

The diagnosis of OA can be made by joint symptoms, radiographic changes, or a combination of the two (Menz et al. 2007). It is, however, very common that a patient with clear radiographic changes specific for OA is totally asymptomatic. This radiographic OA represents the majority of the population prevalence of OA and rather than being a disease, it is even suggested to be part of normal aging in humans (Dieppe 2005). It is not known why pain symptoms are initially provoked in certain individuals with radiographic OA. Nor is it known why among the individuals with symptomatic OA, the structural severity of the disease usually correlates poorly with the intensity of the symptoms, making the attempts for treatment as well as evaluation of the disease outcome in clinical trials difficult (Kean et al. 2004, Dieppe 2005). Furthermore, it is noteworthy that according to epidemiologic follow-up studies, the experiencing of symptoms, particularly pain, is likely to be a risk factor for the progression of OA (Dougados 2004).

**OA of the hand**

For a long time, the focus of research has mainly been on hip and knee OA and as a result, knowledge of the pathogenesis of hand osteoarthritis (HOA) is still limited (Kloppenburg et al. 2007a). Yet, the functional impact of HOA is undisputed as it causes considerable disability in society and is characterized by restricted mobility and motion pain in the hand joints (Kloppenburg 2007b). Magnetic resonance imaging (MRI) has shown that the disease process in HOA includes cartilage loss, bone edema, synovial enhancement, osteophytosis and erosions (Tan et al. 2005). In the late stage, subluxation and fibrotic ankylosis are common, but in some patients, the dominant aspect is bone erosion (Fumagalli et al. 2005). Typical features for HOA are nodules, which may also result from other conditions (Fumagalli et al. 2005). Heberden's nodes are found in the dorsolateral sites of the distal interphalangeal (DIP) joints and are more common than the Bouchard's nodes found in the same sites of proximal interphalangeal (PIP) joints (Alexander 1999). These hard, visible lumps are strongly heritable and are thought to arise from the osteophytes. They may grow slowly and be
painful or painless. The familial incidence of nodes may reflect the inheritance of anatomic characteristics that determine the level of resistance to osteophyte growth. (Alexander 1999, Fumagalli et al. 2005.) Recently, the probability of hand OA was estimated to be 20% when Heberdens nodes are present without any other findings, increasing up to 88% in individuals over 40 years of age having JSN in any finger joint and a family history of nodes (Zhang et al. 2008).

Hand joints comprise 30% of all affected joints in OA (Cushnaghan & Dieppe 1991). HOA is often polyarticular and its specific radiographic changes are most prevalent in the DIP, PIP and the first carpometacarpal (CMC) joints (Wilder et al. 2006). Symptoms of HOA are most commonly experienced in DIP joints (Niu et al. 2003). Symptomatic HOA is less studied, although it is a more relevant indicator of the proportion of patients in need of treatment (Kloppenburg 2007b). Women have a higher prevalence of both radiographic and symptomatic HOA and the prevalence among women also increases with age (Jones et al. 2002, Niu et al. 2003, Oulette & Makowski 2006). The polyarticular nature of HOA is clearly seen in different patterns of joint involvement associated with the disease. The most important patterns in which the joints are affected, in descending order of importance, are symmetry (the same joint affected in opposite hands), clustering by row (the same joint affected in several fingers) and clustering by rays (multiple joints affected in the same finger), which are all seen especially in women (Egger et al. 1995, Chaisson et al. 1997, Niu et al. 2003, Poole et al. 2003, Solovieva et al. 2005, Toba et al. 2006). Unlike in other types of OA, there is a positive association between radiographic HOA and hand pain, as well as between hand pain and the severity of radiographic findings (Dahaghin et al. 2006). Furthermore, OA of the hands may be a risk factor for osteoarthritis of the weight bearing knee and hip joints (Dahagin et al. 2005). (For more detailed reviews, see Fumagalli et al. 2005, Oulette & Makowski 2006 and Kloppenburg 2007b.)

**OA of the hip and knee**

The joints of the hip and knee are the essential weight-bearing joints in humans and their faultless functioning is essential for movement. As this functioning is disturbed by OA, it is likely to raise a more relevant clinical problem to the affected individual than that of HOA. In general, majority of the economic burden of OA is caused by the disabilities resulting from the affected hip and knee joints (Loza et al. 2009). The symptoms and clinical findings present in hip and knee OA include all of those described earlier in section 2.3.1.
Characteristic to both of these conditions is the reduced ability for joint movement (extension-flexion and also rotations in hip OA) and persistent pain that typically occurs during walking, running, climbing stairs and while standing up. Knee OA most commonly affects the femoro-tibial joint and the pain symptoms are mainly restricted to the areas in and around the joint. In hip OA, the localization of symptoms is often not as specific and may be experienced widely around the upper thigh area (O’Reilly & Doherty 2003). At least concerning the knee joint, there seems to be a significant discordance between the clinical outcome and radiological findings of OA (Hannan et al. 2000, Toivanen et al. 2007).

### 2.3.2 Prevalence of osteoarthritis

A majority of the individuals over the age of 65 have radiographic and/or clinical evidence of OA, underlining the importance of aging behind the increasing prevalence of the disease (Goldring & Goldring 2006). Due to the endemic nature of OA, diverse clinical symptoms, and variability in the radiologic classification criteria, it is difficult to compare the exact prevalence of OA between different populations and subgroups. Hence, the frequency of OA in a general population is not well established. Recently, in a survey of a health care database including 4 million people, the overall prevalence of OA was reported to be 10.8%: 8.9% in men and 12.6% in women (the diagnosis of OA was based on international classification of diseases (ICD)) (Kopec et al. 2007). When evaluating the prevalence of OA, it is important to distinguish between radiographic OA and symptomatic OA, the latter being less studied but clinically more relevant.

Joints of the hands, hips and knees are most commonly affected by OA (Goldring & Goldring 2006). According to pathological studies, OA specific changes are present in the knees of 60% of men and 70% of women who die in the seventh and eighth decades of life (Arden & Nevitt 2006). Radiographic knee OA is less frequent than hand disease. A survey of radiographic hand OA in a population of 40 years and older reported the prevalence rates for the second DIP joint, third PIP joint and first CMC joint to be 35%, 18% and 21%, respectively (Wilder et al. 2006). Among Finns aged 30 or older, the prevalence of radiographic OA in any finger joint and in at least two symmetrical pairs of DIP joints was estimated to be 44.8% and 16.0%, respectively (Haara et al. 2003). In the CMC joint of the thumb the prevalence was 7% for men and 15% for women (Haara et al. 2004). In general, population-based studies in the US and Europe
suggest the prevalence rate of severe radiographic disease among people aged 25–34 to be 1%, rising up to 30% in those aged 75 and above. In contrast, only 3–5% of the elderly population have radiographic hip OA making it clearly less common. (See Arden & Nevitt 2006.)

The prevalence of symptomatic OA in general is lower than that of radiographic OA. In the Australian population, it occurs in fewer than 5% of people under the age of 40, increasing somewhat linearly to more than 50% of women and more than 30% of men aged 85 years (March & Bagga 2004). The estimated prevalence of symptomatic OA in different populations is presented in Table 1. A variation in the prevalence rates according to geographical origin of the population is apparent. It is supported by the finding that symptomatic knee OA was more common in Chinese women than in American women (Zhang et al. 2001) and that the prevalence of hip OA significantly varies even within Scandinavia (Ingvarsson 2000).

In summary, the prevalence of symptomatic OA varies between different epidemiological studies, mostly due to different methodological assessments, the geographical origin of the population and discrepancies in defining OA. Still, the prevalence rate in general has been shown to be substantial, demonstrating the clinical burden caused by the different forms of this chronic disease.

Table 1. Estimated prevalence of symptomatic OA of the hand, hip and knee in different populations.

<table>
<thead>
<tr>
<th>Type of OA</th>
<th>Age group (years)</th>
<th>Sex</th>
<th>Prevalence (%)</th>
<th>Population</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand</td>
<td>&gt; 60</td>
<td>F+M</td>
<td>8</td>
<td>US</td>
<td>Dillon et al. 2007</td>
</tr>
<tr>
<td>Hand</td>
<td>&gt; 65</td>
<td>F+M</td>
<td>5.6</td>
<td>Greek</td>
<td>Andrianakos et al. 2006</td>
</tr>
<tr>
<td>Knee</td>
<td>≥ 30</td>
<td>M</td>
<td>6.1</td>
<td>Finnish</td>
<td>Kaila-Kangas 2007</td>
</tr>
<tr>
<td>Knee</td>
<td>≥ 30</td>
<td>F</td>
<td>8.0</td>
<td>Finnish</td>
<td>Kaila-Kangas 2007</td>
</tr>
<tr>
<td>Knee</td>
<td>&gt; 45</td>
<td>M</td>
<td>13.5</td>
<td>US</td>
<td>Jordan et al. 2007</td>
</tr>
<tr>
<td>Knee</td>
<td>&gt; 45</td>
<td>F</td>
<td>18.7</td>
<td>US</td>
<td>Jordan et al. 2007</td>
</tr>
<tr>
<td>Knee</td>
<td>40–75</td>
<td>F+M</td>
<td>7.6</td>
<td>French</td>
<td>Roux et al. 2007</td>
</tr>
<tr>
<td>Knee</td>
<td>&gt; 65</td>
<td>F+M</td>
<td>20.1</td>
<td>Greek</td>
<td>Andrianakos et al. 2006</td>
</tr>
<tr>
<td>Hip</td>
<td>≥ 30</td>
<td>M</td>
<td>5.7</td>
<td>Finnish</td>
<td>Kaila-Kangas 2007</td>
</tr>
<tr>
<td>Hip</td>
<td>≥ 30</td>
<td>F</td>
<td>4.6</td>
<td>Finnish</td>
<td>Kaila-Kangas 2007</td>
</tr>
<tr>
<td>Hip</td>
<td>40–75</td>
<td>F+M</td>
<td>5</td>
<td>French</td>
<td>Roux et al. 2007</td>
</tr>
<tr>
<td>Hip</td>
<td>&gt; 65</td>
<td>F+M</td>
<td>2.5</td>
<td>Greek</td>
<td>Andrianakos et al. 2006</td>
</tr>
</tbody>
</table>
2.3.3 Etiopathogenesis of osteoarthritis

OA is considered to be a heterogeneous group of distinct diseases characterized by an adaptive response of synovial joints to a variety of environmental, genetic, and biomechanical stresses (Haq et al. 2003). According to the current knowledge, the risk factors of the disease relate to one of the two fundamental mechanisms in OA development: the harmful effect of abnormal loading on normal cartilage, or normal loading on abnormal cartilage. A schematic depicting the factors that are believed to be essential for the onset of OA are presented in Figure 6.

Fig. 6. Etiopathogenesis of OA.

Risk factors

Aging is the primary risk factor for OA and it represents normal loading on abnormal cartilage (Heliövaara et al. 1993, Tepper & Hochberg 1993, Hart et al. 1999, Karlson et al. 2003). Aging cartilage loses its tensile strength as the stiffness of the matrix decreases and the articular surface begins to soften. This is thought to derive from the changes in the structural composition of the ECM, particularly changes in content and organization of collagen II and proteoglycans. The capacity of chondrocytes to remodel and repair cartilage ECM also diminishes with age. (Goldring & Goldring 2007.)

Obesity is one of the best studied risk factors for OA (Heliövaara et al. 1993, Felson et al. 1997, Lievense et al. 2002, Cooper et al. 2000). Cartilage disruption is promoted by the increased dynamic stress and abnormal loading that the joints
need to support when overweight, and it is a risk factor particularly for bilateral knee and hip OA. Obese individuals also have a higher bone mass, which may contribute to cartilage breakdown, increasing the stiffness of the subchondral bone. Interestingly, obesity is also associated with HOA. This suggests that there may be a systemic factor related to obesity, which affects the risk of OA.

Important risk factors also include a continuous physical work load caused by occupational factors or sporting activities (Heliövaara et al. 1993, Kujala et al. 1994, Sandmark et al. 2000, Lievense et al. 2001). Fractures and other injuries of the limbs are linked to hip and knee OA in cross-sectional and case-control studies and constitute an important risk factor for secondary OA (Heliövaara et al. 1993, Lau et al. 2000, Wilder et al. 2002).

In addition, a combination of multiple genetic factors constitutes an important risk factor group for OA. Heritability of OA is discussed in more detail in section 2.4.

Women are generally more frequently affected by OA than men, and gender is thus considered to be an important risk factor. Sexual hormones may be involved in the pathogenesis because the occurrence of OA and joint symptoms increase at menopause. There is also some evidence for a protective effect of estrogen in OA. Results of the cohort and case-control studies are not unanimous, but it is likely that estrogen replacement therapy (ERT) has a modest protective effect against hip and knee OA. Furthermore, as a minor risk factor, joint laxity may cause malalignment of the joint components, thus predisposing to OA. More recently, metabolic factors such as high plasma glucose level have also been suggested to affect OA pathogenesis, probably by causing changes in matrix macromolecules. (For more detailed reviews of the risk factors, see Carman et al. 1994, Cicuttini et al. 1996, Cimmino & Parodi 2005, Bierma-Zeinstra & Koes 2007, Fontana et al. 2007 and Riyazi et al. 2008).

The disease process and molecular changes in OA cartilage

Pathological cartilage breakdown in OA is a result of the imbalance between the catabolic (matrix degradation) process and the anabolic attempts at repair (matrix biosynthesis) by chondrocytes (Laadhar et al. 2007). Three stages have been identified in the process, first, the water content of AC increases and the matrix network degrades on a molecular level. Aggrecan size and content decrease and the collagen network is damaged, leading to reduced stiffness of the cartilage. Secondly, the chondrocytes try to compensate the occurred damage by increasing
metabolic activity and matrix biosynthesis. Finally, the chondrocytes can no longer maintain the repair activity and the result is a complete loss of cartilage tissue. (Lorenz & Richter 2006.) As OA advances, the water content of AC is decreased along with the overall proteoglycan content. Chondrocytes become unable to maintain the collagen network and the catabolism and cleavage of proteoglycans and collagen II is further enhanced by the proteolytic enzymes. The changes in the proteoglycan components seem to occur before the collagen network is disrupted and are likely to result from proteolytic cleavage of the native molecules (Horton et al. 2006).

Matrix degrading enzymes such as MMPs and aggrecanases are involved in the disease process. Their synthesis is affected by proinflammatory cytokines, including different interleukins (ILs) and tumor necrosis factor (TNF) –α that are produced originally by the synovium (Martel-Pelletier 1998). Chondrocytes have receptors, including integrins that are activated from the binding of fibronectin and collagen II fragments and in turn stimulate the production of proteinases, inflammatory cytokines and chemokines (Pulai et al. 2005, Xu et al. 2007). Thus, the remains of cleaved matrix components seem to play a role in further inducing degradation of cartilage. In OA, ECM matrix synthesis is also stimulated by different anabolic factors. These include insulin-like growth factor (IGF)-1, TGF-β, fibroblast growth factors (FGFs) and bone morphogenetic proteins (BMPs) (Lorenz & Richter 2006).

In early or mild OA, biochemical analyses have shown that both collagen and proteoglycan synthesis as well as their degradation are upregulated (Lippiello et al. 1977, Sandy et al. 1984, Carney et al. 1992, Squires et al. 2003). Collagen II is downregulated in the upper regions of AC and normally minor components, collagens I and III, are upregulated (Young et al. 2005). Also expression of collagen VI is increased in the lower middle and upper deep zones of AC (Hambach et al. 1998). In addition, collagen X is expressed as a sign of hypertrophy (Aigner et al. 1993).

Regulation of noncollagenous components of the matrix is also affected in early OA. Fibronectin, tenascin, COMP, MMPs including collagenase-1 and -3, and CILP are all upregulated (Fernandes et al. 1998, Wagner et al. 2003, Lorenzo et al. 2004). Also, gene expression analyses of aggrecan and SLRPs have shown upregulation in early OA (Young et al. 2005). In healthy cartilage, there is a homeostasis between MMPs and tissue inhibitors of metalloproteinases (TIMPs), which is disturbed in OA. The activities of MMPs are controlled by TIMPs and the expression of both molecule families has been observed to change in OA.
Particularly the increase in MMP-13 activity is thought to be involved in cartilage degeneration via effective degrading of collagen II. (Lorenz & Richter 2006.) In addition, aggrecanases (ADAMTSs), members of the MMP family, have been shown to contribute in the pathological process of OA (Glasson et al. 2005) by cleaving the important proteoglycan aggregates. It is also possible that the loss of AC in OA reflects the insufficiency of anabolic growth factors, such as IGF-1 and TGF-β, and that inhibition of the growth factors may contribute to the development of the disease (Trippel 2004).

In addition to the proteolytic enzymes, the destructive process involves nitric oxide (NO); it is produced in osteoarthritic joints, where it mediates the destructive effects of different proinflammatory cytokines (Vuolteenaho et al. 2007.) It is notable that the changes in the expression and synthesis of various ECM molecules during OA differ between the zones of AC, as they do also in healthy cartilage. Many of the changes are likely to represent attempts to repair the emerging damage, while the continuing alteration of matrix components in advanced OA demonstrates the break-down of these repair mechanisms. To date, many aspects of the molecular pathology of OA have been revealed and the extensive amount of knowledge only underlines the complexity of the disease process. The underlying trigger of cartilage degradation and the different pathways involved remain to be found in future studies. (For reviews, see Martel-Pelletier 2004, Trippel 2004, Lorenz & Richter 2006 and Goldring & Goldring 2007.)

2.3.4 Relevance of inflammation in osteoarthritis

OA has been classified as a noninflammatory arthritis, partly due to the low amount of leukocytes found in the synovial fluid, and because of the lack of systemic manifestations of inflammation. Nevertheless, the clinical outcome of OA with pain, swelling, effusions and stiffness clearly reflects inflammation (Krasnokutsky et al. 2007). Today, there is mounting evidence that inflammation of joint synovium and AC at the molecular level plays an important role in the pathogenesis of OA, even though it is not usually seen as extensive as in classic inflammatory arthritis, such as RA (Pelletier et al. 2001, Bonnet & Walsh 2005, Kristoffersen et al. 2006).

The mechanism by which production of inflammatory mediators is initiated is yet unclear (Goldring & Goldring 2007). It has been suggested that the inflammation of synovium develops due to the breakdown of matrix, which is
catalyzed by proteolytic enzymes. As a result of the release of increased amounts of matrix fragments into the fluid, inflammatory mediators are created by the synovium. This causes a vicious cycle with more cartilage being degraded, subsequently provoking more inflammation (Martel-Pelletier 2004). From the synovial membrane, inflammatory mediators are able to diffuse through the synovial fluid into cartilage and activate chondrocytes (Martel-Pelletier 1998). Abnormal mechanical forces also seem to activate chondrocytes to produce different inflammatory mediators, such as cytokines, chemokines, leukemia inhibitory factor (LIF) and reactive oxygen species (ROS) including NO. These factors along with lipid-derived inflammatory mediators, such as prostaglandins and leukotrienes, provoke cells to produce MMPs and aggrecanases that may ultimately cause destruction of the ECM (for reviews, see Loeser 2006).

**Cytokines**

At present, cytokines can be regarded as diverse molecules including interferons, interleukins, mesenchymal growth factors, adipokines, and the TNF and chemokine protein families. Cytokines affect nearly every biological process, including responses to infection and antigens, embryonic development, disease pathogenesis and aging. Some of them are primarily lymphocyte growth factors, some function as proinflammatory or anti-inflammatory molecules, whereas other cytokines mediate the immune responses to antigens. (Dinarello 2007.) Cytokines bind to high affinity cell surface receptors, which mediate their actions (Martel-Pelletier 2004). To date, there are 33 known ILs among the cytokines. ILs can be classified into proinflammatory (IL-1, IL-11, IL-17, IL-18), proinflammatory/regulatory (IL-6 and IL-8) and anti-inflammatory (IL-4, IL-10 and IL-13) cytokines (Malemud 2004).

Proinflammatory cytokines are able to affect chondrocytes in various ways. They can increase enzyme synthesis and inhibit the synthesis of important matrix components, including collagens and proteoglycans, as well as reduce the synthesis of their own physiological inhibitors (Martel-Pelletier 2004). Particularly IL-1β and TNF-α seem to be the key mediators in joint destruction. In OA cartilage, chondrocytes synthesize IL-1β at concentrations that can evoke the expression of MMPs, aggrecanases and other catabolic genes. IL-1β molecules colocalize with TNF-α, MMPs and collagen II cleavage epitopes in the damaged matrix. (Goldring & Goldring 2007.) IL-1β and TNF-α also decrease collagen synthesis and stimulate the expression of other inflammatory mediators, such as
IL-6, IL-8, IL-17, IL-18, LIF, prostaglandin E2 and NO (Goldring & Goldring 2007, Krasnokutsky 2007).

IL-1β is synthesized as an inactive precursor, which is activated through protease cleavage by IL-1β converting enzyme (ICE or caspase-1) (Fernandes et al. 2002). ICE is produced in the synovial membrane and in the AC and its production is significantly increased in OA tissues (Saha et al. 1999). Biological activities of IL-1 are mediated via binding to two cell-surface receptors, IL-1R1 and IL-1R2 (Slack et al. 1993), and the number of IL-1R1 is reported to be significantly increased in OA (Sadouk et al. 1995). Both types of IL-1R can also be shed from the cell surface, and exist extracellularly in truncated forms known as IL-1 soluble receptors (Fernandes et al. 2002). TNF-α is also synthesized as a precursor and is activated proteolytically by TNF-α converting enzyme (TACE) (Black et al. 1997). TACE has been found to be upregulated in OA (Attur et al. 2002). TNF-α binds to two specific cell membrane receptors and the one has been observed to multiply in OA chondrocytes (Malemud 2004). The actions of proinflammatory cytokines, IL-1β and TNF-α, are regulated in three different ways: (i) their activity can be blocked by a receptor antagonist, such as IL-1Ra; (ii) the soluble receptor binds the free cytokine, thus blocking its activity; or (iii) an anti-inflammatory cytokine decreases the synthesis of IL-1β and TNF-α (Martel-Pelletier 2004).

IL-6 is also believed to be one of the major factors in joint destruction, being a pleiotropic proinflammatory cytokine that is markedly upregulated at times of tissue inflammation. It is believed to be an important contributor to the pathogenesis of OA as it is able to increase the amount of inflammatory cells in synovial tissue, amplify the effects of IL-1 on the increased MMP synthesis and decreased proteoglycan production, as well as stimulate chondrocyte proliferation (Martel-Pelletier 1998). Human recombinant (hr) IL-6 has been shown to enhance hrIL-1β-induced proteoglycan degradation and to inhibit chondrocyte proliferation (Jikko et al. 1998). Furthermore, a significant rise in the level of IL-6 mRNA has been detected in OA-affected cartilage, and IL-6 levels in the serum and synovial fluid have been reported to be elevated among OA patients (Kaneko et al. 2000).

Other cytokines involved in the inflammation reaction in OA are IL-8, IL-17, IL-18 and LIF (Malemud 2004). Natural inhibitors, which reduce the production of proinflammatory cytokines and/or their activity, include TGF-β and the anti-inflammatory cytokines IL-4, IL-10 and IL-13. (For reviews, see Martel-Pelletier
2.4 Inheritance of osteoarthritis

The concept of OA as a hereditary disease began to form in the 1940s, when familial clustering of Heberdens nodes of the fingers was discovered (Stecher 1941). Replication and widening of this study by others provided more understanding of the genetic component of OA, and later it became apparent that the disease is not caused by a single gene, but is rather transmitted in a nonmendelian manner as a complex, multifactorial trait. (For reviews, see Peach et al. 2005.) Comprehensive investigation on the subject was not undertaken significantly until the mid-1990s, when several twin-pair, sibling-risk and segregation studies were conducted. Studies from the past decade have estimated the influence of genetic factors on radiographic OA in women to be between 39% and 65% concerning hand joints and around 60% for the hip joint (Spector & MacGregor 2004). It has also been shown that the degree of heritability between different joint sites and between the sexes may differ, suggesting heterogeneity in the nature of the encoded susceptibility (Loughlin 2005).

Earlier, it was commonly thought that OA is a generalized systemic disease that has a major genetic component. However, this concept of the disease did not provide expected breakthroughs and it has been suggested that a joint-specific and gender-specific approach may be more rewarding (Loughlin 2004). Today, there is further evidence that multiple genes involved in OA pathogenesis may work differently in men and women, as well as affect different joint sites and cause distinct disease phenotypes in terms of severity. This notion is supported by the fact that statistical evidence in favor of chromosomal regions or independent genes often increases when the studied patient cohorts are stratified into subgroups based on sex, affected joint site and severity of the disease (Bukulmez et al. 2006). However, these stratifications often result in insufficient sample sizes with limited statistical power, which is likely to increase the possibility of false positive findings.

The field of complex genetic diseases has experienced a major revolution during the past few years. This is mostly due to the advanced technology which enables fast and cost-effective analysis of multiple SNPs simultaneously (van der Helm-van Mil et al. 2008). Traditionally, investigators have sought to identify genes that affect OA susceptibility using two different approaches, candidate gene
association analysis and genome-wide linkage analysis. Furthermore, the completion of the human genome project has produced a third and perhaps the most promising method at the moment: genome-wide association (GWA) studies. These methods and their contributions to OA genetics are reviewed in more detail later in sections 2.4.1 and 2.4.2. Finally, a new alternatively used strategy is gene expression analysis, which is targeted to identify genes that are significantly up- or downregulated in OA compared to normal tissue, thus indicating a role in disease development or progression (Loughlin 2005). During the past few years, all of these strategies have provided extensive new information about the inheritance patterns and genome location of potential causative variants; yet, the results have been generally inconsistent at least partly due to the methodological shortcomings described below (Valdes et al. 2006).

Requirements for the quality and quantity of study populations have increased along with the growing data of complex disease genetics. In general, the major portion of the nearly one hundred previously reported different OA-associated variants have been obtained with samples that do not meet all the current standards required for studying a complex trait. If the type 1 error rate is set to a 5% level ($p = 0.05$) in a study of complex disease, in addition to a sufficient sample size, a careful definition of the phenotype is required and the population stratification also needs to be taken into account in order to obtain valid results. Furthermore, possible findings need correction for multiple testing if tested for independent associations (van der Helm-van Mil et al. 2008).

Limited-sized case-control studies of individual variations are laborious and require a hypothesis of the biological function or location of the candidate gene. Linkage analysis, on the other hand, is often not powerful enough to detect common alleles that have low penetrance and low frequency (Hirschhorn & Daly 2005). In contrast, during the past few years many common genetic variations with low penetrance have been successfully shown to associate with OA (Valdes et al. 2008a) as well as to various other complex diseases including cardiovascular, metabolic and autoimmune conditions, using the GWA approach with large (often thousands of individuals) study samples (Frazer et al. 2009). However, unlike in many of the candidate gene association analyses, the meaning of the results obtained by GWA studies are often unclear and, like the candidate analyses, they also require replication in independent case-control studies (Van der Helm-van Mil et al. 2008).
2.4.1 Association analyses of candidate genes

In association analysis, the genotype and allelic distribution of a certain sequence variation is compared between healthy and OA affected individuals. Concerning OA, the genes of interest have most often included those that code for the important structural proteins of AC, such as collagens and proteoglycans (Reginato & Olsen 2002). Also the genes of different noncollagenous proteins and regulatory and signalling molecules of AC have been widely screened for association with OA (Spector & MacGregor 2004). Taken together, numerous candidate gene studies performed in different geographical populations and OA subsets worldwide have yielded dozens of statistically significant associations within a variety of different genes (Bukulmez et al. 2006). To date, only a few of the positive findings have been successfully replicated in an independent population, and most of the associated variations still lack solid evidence for causality and functional differences between susceptibility alleles. (For a review, see Ikegawa 2007.) The genetic variations that have shown the most compelling evidence of association in different studies to date are summarized in Table 2.

Despite the reported associations, the role of major structural genes, particularly \textit{COL2A1} and \textit{AGC1}, in OA is still unclear. SNP haplotypes within \textit{COL2A1} have been demonstrated to associate with OA in two different ethnic groups (Meulenbelt et al. 1999, Ikeda et al. 2002) and were replicated more recently (Valdes et al. 2007, Hämäläinen et al. 2008). Still, the function of the associated haplotypes is unknown and in general, the findings do not support the idea of \textit{COL2A1} as a major susceptibility gene for the common form of OA (Jakkula et al. 2005). Also, the functional VNTR polymorphism within the \textit{AGC1} gene has been shown to associate with OA in two independent samples, but the results have been inconsistent (Horton et al. 1998, Kirk et al. 2003).

The asporin gene (\textit{ASPN}) and the secreted frizzled-related protein 3 gene (\textit{FRZB}) are susceptibility genes for which the disease causality and functional differences in disease alleles have been convincingly shown (Loughlin et al. 2004, Kizawa et al. 2005). Asporin belongs to the SLRP family and is considered to be important in mediating cartilage metabolism through binding to TGF-\(\beta\). \textit{ASPN} contains a unique aspartic acid (D) repeat in its N-terminal region. The D14 allele of this polymorphic site has been shown to inhibit TGF-\(\beta\) activity more than other alleles, and was recently found to be overrepresented in two independent Japanese osteoarthritic populations (Kizawa et al. 2005). Thus, this variation seems to have functional significance and it provides a link between ECM proteins, TGF-\(\beta\)
activity and the disease. To date, the association has been confirmed in various independent populations (Mustafa et al. 2005, Jiang et al. 2006, Shi et al. 2007). Some of the recent studies have not confirmed the association, but in contrast, have suggested the role of D13 as a protective allele (Kaliakatsos et al. 2006, Valdes et al. 2007, Song et al. 2008). Also, a total lack of association has been reported in some ethnic groups (Rodriguez-Lopez et al. 2006, Atif et al. 2008, Limer et al. 2008). In conclusion, a meta-analysis suggested that the association between the ASPN D14 allele and knee OA has global relevance, but the effect seems to have ethnic differences (Nakamura et al. 2007).

There are two single nucleotide polymorphisms (SNPs) in the area of the FRZB gene that encode for a substitution of highly conserved arginine residues to either tryptophan (Arg200Trp) or glycine (Arg324Gly) and result in reduction in the ability of the mature protein to antagonize Wnt signalling, which is crucial for chondrocyte metabolism (Loughlin 2005). These SNPs have been repeatedly shown to associate or suggested to associate with OA in different ethnic populations (Loughlin et al. 2004, Min et al. 2005, Lane et al. 2006, Valdes et al. 2007 and Rodriguez-Lopez et al. 2007). Together with the functional causality, the association studies provide evidence of the contribution of FRZB in the pathogenesis of OA, although they could not be confirmed in a recent meta-analysis (Kerkhof et al. 2008).

Another promising susceptibility gene for OA is growth and differentiation factor 5 (GDF5), which belongs to the BMP gene family. An individual SNP within the gene may reduce the transcriptional activity of chondrogenic cells, and to date it has been shown to associate with OA in three distinct Asian populations (Miyamoto et al. 2007). The association has also been confirmed in European populations, and the SNP was reported to be functional, causing decreased expression of the gene (Southam et al. 2007, Vaes et al. 2008, and Valdes et al. 2008).

The matrilin 3 gene (MATN3) was originally identified as an OA susceptibility gene through a genome wide linkage scan, and a missense Thr303Met mutation in the gene has repeatedly been reported to increase the risk and severity of HOA (Stefánsson et al. 2003, Eliasson et al. 2006, Pullig et al. 2007). Furthermore, a disintegrin and metalloproteinase gene, ADAM12, important in mediating cellular interactions and responses, has been seen to strongly associate with knee OA in two separate populations (Valdes et al. 2004, 2006).
At present, the role of cytokines in the genetics of OA is unclear. Markers within the IL-1 gene cluster have earlier shown moderate evidence of association with knee OA (Loughlin et al. 2002a). Further evidence of this association was more recently gained by haplotype analyses in two small independent cohorts (Meulenbelt et al. 2004, Smith et al. 2004). Confusingly, neither one of the findings could be confirmed in a population that was more than six times larger and comparable in terms of ethnicity and disease classification (Chapman & Loughlin 2006). Yet, the SNPs and different haplotypes in the IL-1 gene cluster have repeteadly been positively linked to knee and hand OA also in other populations (Stern et al. 2003, Moxley et al. 2007 and Kanoh et al. 2008), further implying that there are likely one or more genetic features within the area that contribute in the etiology of OA at some level. Also, contradictory results have been reported (Ni et al. 2009). Furthermore, association of variants within the interleukin 4 receptor (IL-4R), IL-6 and IL-10 genes have also been reported (Forster et al. 2004a, Pola et al. 2005, Fytili et al. 2005, Riyazi et al. 2005), but the findings still lack confirmation in independent samples. In summary, the results concerning the IL-1 gene cluster in particular are diverse, consisting of multiple susceptible alleles and haplotypes. More research is needed in order to accurately define the genetic effect of the common inflammatory mediators on OA susceptibility.

There is also evidence of the role of the vitamin D receptor (VDR) gene in OA. Polymorphisms in the gene that are detectable with TaqI and ApaI restriction enzymes have been shown to associate with knee and hand OA (Keen et al. 1997, Utterlinden et al. 1997 and 2000, Solovieva et al. 2006). Nevertheless, many attempts to reproduce these associations have failed (Huang et al. 2000, Baldwin et al. 2002, Valdes et al. 2006, Limer et al. 2008). Furthermore, no association was confirmed in a recent meta-analysis between the VDR TaqI or ApaI polymorphisms and OA susceptibility (Lee et al. 2008).
Table 2. A summary of genes and underlying variants that have shown convincing association with OA in multiple studies, conducted among populations with different phenotypes and ethnic backgrounds.

<table>
<thead>
<tr>
<th>Gene (Locus)</th>
<th>Variation</th>
<th>OA*</th>
<th>Sex</th>
<th>Cases (n) / Controls (n)</th>
<th>Minor allele frequency % cases / controls</th>
<th>OR</th>
<th>p-value</th>
<th>Population</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASPN (9q22.31)</td>
<td>Allele D14</td>
<td>K/M</td>
<td>137 / 234</td>
<td>10.9 / 4.7</td>
<td>2.63 0.00084</td>
<td>Japanese</td>
<td>Kizawa et al. 2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Allele D14</td>
<td>H/M</td>
<td>593 / 374</td>
<td>7.9 / 4.8</td>
<td>1.70 0.0078</td>
<td>Japanese</td>
<td>Kizawa et al. 2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Allele D14</td>
<td>H/M</td>
<td>364 / 356</td>
<td>17.4 / 13.1</td>
<td>1.48 0.016</td>
<td>British</td>
<td>Mustafa et al. 2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Allele D14</td>
<td>K/M</td>
<td>218 / 454</td>
<td>9.4 / 4.9</td>
<td>2.04 0.0013</td>
<td>Chinese</td>
<td>Jiang et al. 2006</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Allele D14</td>
<td>K/M</td>
<td>354 / –</td>
<td>8.8 / –</td>
<td>na 0.004</td>
<td>Chinese</td>
<td>Shi et al. 2007a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRZB (2q32.1)</td>
<td>Arg324Gly</td>
<td>H/F</td>
<td>378 / 760</td>
<td>11.0 / 7.0</td>
<td>1.5 0.04</td>
<td>British</td>
<td>Loughlin et al. 2004</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg200Trp and Arg 324Gly</td>
<td>H/F</td>
<td>558 / 760</td>
<td>2.6 / 0.6</td>
<td>4.1 0.007</td>
<td>British</td>
<td>Loughlin et al. 2004</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg324Gly</td>
<td>G/F</td>
<td>545 / 1362</td>
<td>11.0 / 8.0</td>
<td>1.6 0.02</td>
<td>Dutch</td>
<td>Min et al. 2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg200Trp and Arg 324Gly</td>
<td>H/F</td>
<td>570 / 1317</td>
<td>5.0 / 2.0</td>
<td>1.90 0.01</td>
<td>US</td>
<td>Lane et al. 2006</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg200Trp and Arg 324Gly</td>
<td>K/F</td>
<td>603 / 599</td>
<td>2.0 / 0.7</td>
<td>2.87 0.04</td>
<td>Caucasian</td>
<td>Valdes et al. 2007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GDF5 (20q11.22)</td>
<td>C+104T</td>
<td>K/F</td>
<td>718 / 861</td>
<td>21.2 / 25.9</td>
<td>1.30 0.0021</td>
<td>Japanese</td>
<td>Miyamoto et al. 2007</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C+104T</td>
<td>H/F</td>
<td>1000 / 981</td>
<td>16.0 / 26.0</td>
<td>1.79 1.8x10^{-13}</td>
<td>Japanese</td>
<td>Miyamoto et al. 2007</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C+104T</td>
<td>K/F</td>
<td>313 / 485</td>
<td>21.6 / 29.8</td>
<td>1.54 0.00028</td>
<td>Chinese</td>
<td>Miyamoto et al. 2007</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C+104T</td>
<td>K/H</td>
<td>2487 / 2018</td>
<td>12.8 / 15.9</td>
<td>1.28 0.004</td>
<td>Spanish UK</td>
<td>Southam et al. 2007</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C+104T</td>
<td>K/H</td>
<td>1842 / 1166</td>
<td>32.4 / 40.5</td>
<td>1.29 8.8x10^{-4}</td>
<td>UK</td>
<td>Valdes et al. 2008</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C+104T</td>
<td>H/F</td>
<td>604 / 1102</td>
<td>na</td>
<td>0.68 0.8x10^{-5}</td>
<td>Caucasian</td>
<td>Vaes et al. 2008</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C+104T</td>
<td>K/F</td>
<td>494 / 1174</td>
<td>na</td>
<td>0.68 0.003</td>
<td>Caucasian</td>
<td>Vaes et al. 2008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MATN3 (2p24.1)</td>
<td>Thr303Met</td>
<td>H/F</td>
<td>2162 / 873</td>
<td>2.1 / 1.0</td>
<td>2.12 na</td>
<td>Iceland</td>
<td>Stefánsson et al. 2003</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thr303Met</td>
<td>H/F</td>
<td>226 / 356</td>
<td>10 / 2.5</td>
<td>4.28 0.007</td>
<td>German</td>
<td>Pullig et al. 2007</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Association for early onset OA; *G = generalized; Ha = hand; K = knee; H = hip; na = not available.
2.4.2 Genome-wide studies

Another approach to OA genetics has been genome wide linkage analyses that aim to uncover chromosomal regions predicted to contain variants that contribute to OA pathogenesis. Traditionally, these studies have been conducted using cohorts of affected sibling pairs or families where OA is exceptionally prevalent and seems to be inherited in a Mendelian manner.

Two different genomic loci are considered to be in linkage with each other if they are transmitted together from parent to offspring more frequently than would be probable with independent inheritance (Schork 1997). A large amount of polymorphic microsatellite markers consisting of repeating units typically 2–4 base pairs in length have been identified in the genome and their inheritance can be accurately monitored (Goldstein et al. 2005). These microsatellite repeats are usually not associated with any clinical trait and are called genetic markers. If a genetic marker is situated close enough to a locus that harbors a disease-associated variant, it is likely that they are inherited without a recombination occurring between them during meiosis and are thus linked. Analyses aiming to identify new susceptibility loci for OA can be conducted by genotyping polymorphic microsatellite markers and following their segregation through pedigrees with multiple OA-affected family members.

Since OA is a multifactorial disease with no clear mode of inheritance, non-parametric (model-free) linkage analysis would be expected to yield more accurate results. Parametric analysis requires specification of inheritance model and disease allele frequency. It is more powerful than non-parametric linkage analysis if the penetrance model and disease allele frequencies are estimated correctly (Kruglyak et al. 1996). Parametric and non-parametric linkage analyses can be performed in two ways: two-point and multipoint. In two-point analysis, the probability of linkage (the marker and disease are inherited together more often than expected under Mendelian law) is calculated with different recombination fractions separately between each marker and the disease locus (Aston & Wilson 1986). In multipoint analysis, all markers on the same chromosome are tested jointly for linkage and the position that is in linkage with the disease is the one that maximizes the LOD score. The LOD score is proportional to the strength of the evidence of linkage (traditionally ≥3, p = 0.001 is considered the limit for significance). The LOD score is calculated from the best estimation of the recombination fraction (maximum likelihood ratio) and is
Genome-wide linkage studies have concentrated on HOA (Leppävuori et al. 1999, Demissie et al. 2002, Stefánsson et al. 2003, Hunter et al. 2004) and hip OA (Ingvarsson et al. 2001, Chapman et al. 2002, Loughlin et al. 2002b, Forster et al. 2004b, Southam et al. 2004). These studies have uncovered loci with at least suggestive linkage in most chromosomes. Following the evidence that genes may contribute differently in men and women and vary also according to the site of the joint, recent linkage studies have yielded more convincing results using the preceding stratifications in the analyses (Creig et al. 2006, Livshits et al. 2007). A summary of positive linkage results to date is presented in Table 3, in which the limit for the positive linkage in terms of logarithm of odds (LOD) score is set to 2.0; however, lower suggestive results (LOD > 1.5) are also presented if the locus of linkage is close or overlaps with another suggestive region.

At present, Chr 2 is perhaps the strongest candidate for carrying important OA-associated variants. Regions in its short arm have earlier been linked to HOA (Demissie et al. 2002, Stefánsson et al. 2003) and have led to the identification of \textit{MATN3} as an important candidate gene (Stefánsson et al. 2003). Furthermore, one of the largest OA linkage studies performed to date recently provided strong evidence of linkage (LOD 4.0) at a locus on Chr 2p to HOA (Livshits et al. 2007). There are also loci with significant linkage in the long arm of Chr 2 (Leppävuori et al. 1999, Loughlin et al. 2002b, Hunter et al. 2004), and \textit{FRZB} has been identified as an OA-associating candidate gene in the area (Loughlin et al. 2004).

In an adjacent locus in the telomeric direction from the preceding areas, a strong linkage (LOD 6.1) was obtained within families manifesting early-onset generalized OA (Meulenbelt et al. 2006). The following fine mapping of the area has led to the identification of two potential variants in the \textit{NRP2} and \textit{IDH1} genes (Min et al. 2007). Overall, these results suggest that genomic variants particularly within Chr 2 are important in the development of OA.

Taken together, there are numerous loci within 1q, 3p, 4q, 11q and 12q with suggestive linkage to different phenotypes of HOA and to hip OA (see Table 3). The reported linked regions do not tend to overlap, but mostly lie in relatively close proximity to each other. A strong linkage to HOA in a locus 15q was reported by Hunter et al. (2004) with a LOD-score of 6.3, but the locus has not shown positive linkage in other studies. Furthermore, Chr 16 has multiple loci next to each other that have been suggested to be in linkage with hip or HOA (Ingvarsson et al. 2001, Forster et al. 2004b, Greig et al. 2006). IL4R has been presented in the form of log to the base of 10. (For a more detailed review concerning linkage methodology and concepts, see Dawn Teare & Barrett 2005.)
identified as a candidate gene within the region showing linkage to OA (Forster et al. 2004a). There are new linkage results concerning the long arm of Chr 19 that are of particular interest. Evidence for linkage in 19q was earlier reported by Demissie et al. (2002) and the same locus was recently confirmed by Livshits et al. (2007) with a LOD-score of 4.3. In the most recent genome wide analysis, 14q32.11 was linked to generalized OA with a LOD of 3.03 with a potential susceptibility gene (DIO2) identified within the locus (Meulenbelt et al. 2008).

So far, numerous chromosomal loci among different populations have been suggested to harbor OA susceptibility variants. Fine mapping of areas detected by genome-wide linkage studies have resulted in the identification of many candidate genes, but the following association studies have identified a susceptible variation only in a few cases (Stefánsson et al. 2003, Forster et al. 2004a, Loughlin et al. 2004, see also Min et al. 2007). The inconsistency seen in results gained from the linkage analyses to date suggests that the genetic etiology of OA may be heterogenic among geographical populations. Furthermore, it implies that genetic factors may result in different disease outcomes in terms of joint specificity and severity. As ongoing and future linkage studies are performed in even more carefully selected populations, it is possible that they will provide information that will lead to the identification of new important candidate genes for OA.

Table 3. A summary of chromosomal areas that have been reported to be in linked (LOD-score > 2.0) to different OA outcomes. Areas with suggestive linkage (LOD > 1.5) are also presented if they overlap or are in close proximity to another suggestive locus.

<table>
<thead>
<tr>
<th>Chr locus</th>
<th>cM</th>
<th>LOD</th>
<th>Phenotype</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p32.2</td>
<td>102</td>
<td>3.0</td>
<td>JSN in hand</td>
<td>USA</td>
<td>Demissie et al. 2002</td>
</tr>
<tr>
<td>1q31.1</td>
<td>202</td>
<td>3.0</td>
<td>DIP (women)</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>1q32.1</td>
<td>218</td>
<td>2.1</td>
<td>DIP</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>1q 42.1</td>
<td>250</td>
<td>3.0</td>
<td>Hand Tot-KL</td>
<td>UK</td>
<td>Livshits et al. 2007</td>
</tr>
<tr>
<td>2p23.3–24.1</td>
<td>42–47</td>
<td>4.4</td>
<td>DIP/CMC1</td>
<td>Iceland</td>
<td>Stefánsson et al. 2003</td>
</tr>
<tr>
<td>2p23.3</td>
<td>48</td>
<td>2.2</td>
<td>JSN in hand</td>
<td>USA</td>
<td>Demissie et al. 2002</td>
</tr>
<tr>
<td>2p13.2–2p14</td>
<td>90</td>
<td>2.9</td>
<td>DIP</td>
<td>UK</td>
<td>Livshits et al. 2007</td>
</tr>
<tr>
<td>2p12–13.3</td>
<td>95</td>
<td>4.0</td>
<td>Hand Tot-KL</td>
<td>UK</td>
<td>Livshits et al. 2007</td>
</tr>
<tr>
<td>2q12–21</td>
<td>116–155</td>
<td>2.3</td>
<td>DIP</td>
<td>Finland</td>
<td>Leppävuori et al. 1999</td>
</tr>
<tr>
<td>2q31.1</td>
<td>181</td>
<td>1.6</td>
<td>Thumb IP</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>2q24.3–31.1</td>
<td>175–184</td>
<td>1.6</td>
<td>Hip</td>
<td>UK</td>
<td>Loughlin et al. 2002b</td>
</tr>
<tr>
<td>2q33.3</td>
<td>202</td>
<td>6.1</td>
<td>GOA</td>
<td>Netherlands</td>
<td>Meulenbelt et al. 2006</td>
</tr>
<tr>
<td>3p25.1–25.2</td>
<td>30</td>
<td>2.8</td>
<td>DIP</td>
<td>UK</td>
<td>Livshits et al. 2007</td>
</tr>
<tr>
<td>Chr locus</td>
<td>cM</td>
<td>LOD</td>
<td>Phenotype</td>
<td>Country</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
<td>------</td>
<td>-----</td>
<td>--------------------</td>
<td>---------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>3p21.2</td>
<td>71</td>
<td>2.7</td>
<td>PIP</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>3p14.1</td>
<td>91</td>
<td>2.5</td>
<td>Hand JSN &amp; OST</td>
<td>UK</td>
<td>Greig et al. 2006</td>
</tr>
<tr>
<td>3p14.1</td>
<td>96</td>
<td>1.8</td>
<td>DIP</td>
<td>Iceland</td>
<td>Stefánssön et al. 2003</td>
</tr>
<tr>
<td>4p11</td>
<td>57</td>
<td>3.0</td>
<td>JSN in hand</td>
<td>UK</td>
<td>Greig et al. 2006</td>
</tr>
<tr>
<td>4q12–21.2</td>
<td>57–73</td>
<td>3.1</td>
<td>Hip (women)</td>
<td>UK</td>
<td>Loughlin et al. 1999</td>
</tr>
<tr>
<td>4q13.3</td>
<td>79</td>
<td>3.1</td>
<td>Hip</td>
<td>UK</td>
<td>Forster et al. 2004b</td>
</tr>
<tr>
<td>4q 26–27</td>
<td>128</td>
<td>2.3</td>
<td>DIP</td>
<td>Finland</td>
<td>Leppävuori et al. 1999</td>
</tr>
<tr>
<td>4q31.3</td>
<td>154</td>
<td>3.3</td>
<td>DIP</td>
<td>Iceland</td>
<td>Stefánssön et al. 2003</td>
</tr>
<tr>
<td>4q32.3</td>
<td>170</td>
<td>3.8</td>
<td>Hand Tot-KL</td>
<td>UK</td>
<td>Livshits et al. 2007</td>
</tr>
<tr>
<td>6p11.1</td>
<td>78</td>
<td>4.8</td>
<td>Hip (women)</td>
<td>UK</td>
<td>Southam et al. 2004</td>
</tr>
<tr>
<td>6p11.2–12</td>
<td>80</td>
<td>3.1</td>
<td>Hand Tot-KL</td>
<td>UK</td>
<td>Livshits et al. 2007</td>
</tr>
<tr>
<td>6p21.1–q22.1</td>
<td>56–120</td>
<td>2.1</td>
<td>Hip</td>
<td>UK</td>
<td>Loughlin et al. 1999</td>
</tr>
<tr>
<td>7p11.2</td>
<td>50</td>
<td>2.3</td>
<td>JSN in hand</td>
<td>USA</td>
<td>Demissie et al. 2002</td>
</tr>
<tr>
<td>7q35</td>
<td>155</td>
<td>3.1</td>
<td>DIP</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>8p12</td>
<td>61</td>
<td>2.6</td>
<td>JSN in hand</td>
<td>UK</td>
<td>Greig et al. 2006</td>
</tr>
<tr>
<td>8q12–21</td>
<td>105</td>
<td>2.1</td>
<td>DIP</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>9q21.2</td>
<td>76</td>
<td>2.3</td>
<td>JSN in hand</td>
<td>USA</td>
<td>Demissie et al. 2002</td>
</tr>
<tr>
<td>9q34.2–34.3</td>
<td>155</td>
<td>4.5</td>
<td>DIP</td>
<td>UK</td>
<td>Livshits et al. 2007</td>
</tr>
<tr>
<td>10q11.1</td>
<td>63</td>
<td>2.7</td>
<td>First CMC</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>11q13.4</td>
<td>76</td>
<td>1.6</td>
<td>JSN in hand</td>
<td>USA</td>
<td>Demissie et al. 2002</td>
</tr>
<tr>
<td>11q12.1–23.2</td>
<td>72–96</td>
<td>2.4</td>
<td>Hip (women)</td>
<td>UK</td>
<td>Chapman et al. 2002</td>
</tr>
<tr>
<td>12q21.3–22</td>
<td>100</td>
<td>3.9</td>
<td>DIP</td>
<td>UK</td>
<td>Livshits et al. 2007</td>
</tr>
<tr>
<td>12q24.3</td>
<td>166</td>
<td>1.7</td>
<td>JSN in hand</td>
<td>USA</td>
<td>Demissie et al. 2002</td>
</tr>
<tr>
<td>12q24.3</td>
<td>166</td>
<td>1.8</td>
<td>DIP</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>13q33.1</td>
<td>87</td>
<td>2.3</td>
<td>First CMC</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>13q22.1</td>
<td>56</td>
<td>3.57</td>
<td>Hip&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Japan</td>
<td>Mabuchi et al. 2006</td>
</tr>
<tr>
<td>14q32.11</td>
<td>119–123</td>
<td>3.0</td>
<td>GOA</td>
<td>multiple</td>
<td>Meulenbelt et al. 2008&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15q25.3</td>
<td>81</td>
<td>6.3</td>
<td>First CMC</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>16p12.1–13.1</td>
<td>28–49</td>
<td>2.6</td>
<td>Hip&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Iceland</td>
<td>Ingvarsson et al. 2001</td>
</tr>
<tr>
<td>16p 12.2</td>
<td>46</td>
<td>1.7</td>
<td>Hip (women)</td>
<td>UK</td>
<td>Forster et al. 2004b</td>
</tr>
<tr>
<td>16p12.1</td>
<td>52</td>
<td>2.6</td>
<td>JSN in hand</td>
<td>UK</td>
<td>Greig et al. 2006</td>
</tr>
<tr>
<td>16q22.1</td>
<td>89</td>
<td>1.9</td>
<td>Hip (women)</td>
<td>UK</td>
<td>Forster et al. 2004b</td>
</tr>
<tr>
<td>17q13.1</td>
<td>22</td>
<td>2.2</td>
<td>First CMC</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>17q 25.1</td>
<td>100</td>
<td>2.3</td>
<td>DIP</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>19p11</td>
<td>52</td>
<td>1.8</td>
<td>JSN in hand</td>
<td>USA</td>
<td>Demissie et al. 2002</td>
</tr>
<tr>
<td>19q13.2</td>
<td>65</td>
<td>4.3</td>
<td>Hand Tot-KL</td>
<td>UK</td>
<td>Livshits et al. 2007</td>
</tr>
<tr>
<td>19q13.3</td>
<td>68</td>
<td>1.8</td>
<td>Hand Tot-KL</td>
<td>USA</td>
<td>Demissie et al. 2002</td>
</tr>
<tr>
<td>19q13.4</td>
<td>90</td>
<td>4.0</td>
<td>DIP</td>
<td>UK</td>
<td>Livshits et al. 2007</td>
</tr>
<tr>
<td>20p13</td>
<td>4</td>
<td>3.7</td>
<td>DIP (women)</td>
<td>USA</td>
<td>Hunter et al. 2004</td>
</tr>
<tr>
<td>20q11.1</td>
<td>51</td>
<td>2.2</td>
<td>OST in hand</td>
<td>UK</td>
<td>Greig et al. 2006</td>
</tr>
</tbody>
</table>

<sup>a</sup>Linkage study of one family; Associating candidate genes found: <sup>1</sup>MATN3; <sup>2</sup>FRZB; <sup>3</sup>NRP2 and IDH1; <sup>4</sup>DIO2; cM = cent morgan; DIP = distal interphalangeal; GOA = generalized OA; OST = osteophyte; PIP = proximal interphalangeal; JSN = joint space narrowing; Tot-KL = Kellgren-Lawrence score sum for both hands.
The GWA studies enable the comparison of the allele frequencies of a large number of SNPs between cases and controls. The International HapMap Project has resulted in the identification of over 3.1 million SNPs in the human genome with a density of approximately one per kilobase, including approximately 25–35% of all the 9–10 million common SNPs (The International HapMap Consortium 2007). With optimized analysis of these SNPs, most of the common alleles in the entire genome can be covered. The GWA is a more efficient method than the traditional linkage scan and is likely to result in the identification of variations that are very close to or even in the gene that is associated with the disease. Currently, the most up-to-date microarrays, or chips, have a capability of assessing more than 1 million SNPs in a single sample. Nevertheless, this method also has certain weaknesses as pointed out by Hunter & Kraft (2007). Even the GWA method can not cover the variations of the entire genome (false negative) and can also easily lead to false positive results. In order to reduce the possibility of a type 1 error, the study population in GWA analysis needs to be substantially large (preferably thousands of individuals), which is also essential in order to correct for multiple testing and still maintain the statistical significance of the finding (Valdes & Spector 2009).

A case-control study by Mototani et al. (2005) with 72,000 markers found the calmodulin-1 gene (*CALM1*) to be strongly associated with hip OA in a Japanese population (428 cases / 1008 controls, OR 2.40, p = 0.00065). However, the replication attempts in two European populations (920 cases / 752 controls and 158 cases / 193 controls) failed (Loughlin et al. 2006, Poulou et al. 2008, respectively). In a study of more than 25,000 examined SNPs, the leucine-rich repeats and calponin homology domain containing protein 1 gene (*LRCH1*) with an unknown function was reported to associate with radiographic knee OA in a sample of 335 cases and 335 controls (OR 1.44, p = 0.0078) (Spector et al. 2006). Again, a replication attempt in a sufficiently powered independent sample set (1145 cases / 1266 controls) failed (Jiang et al. 2008). One of the newest potential candidate genes is also the previously unknown gene *DVWA* at 3p24.3. It was identified in a recent genome-wide association study and the variations within the gene were shown to increase the risk of knee OA in Asian populations with a combined p-value of $7.3 \times 10^{-11}$ (Miyamoto et al. 2008). One of the reported SNPs in the gene associated with the knee OA also in a large replication study with a population (2602 cases and 2147 controls) of European origin (a meta-analysis with the previous asian populations, OR 1.29, p = $2.70 \times 10^{-5}$) (Meulenbelt et al. 2009).
Perhaps the largest GWA study to date with 500,000 markers, 1564 knee OA cases and 2627 control individuals identified variants within the 2q33 linkage region that associated with knee OA (OR 1.55, p < 6.9 × 10^{-7} Valdes et al. 2008a). This region is currently being tested in a large (n = 8000) consortium and other large cohorts including Rotterdam, Framingham and TwinsUK are also being utilized for GWA analyses (Valdes & Spector 2009).
3 Outlines of the present study

The genetic foundation of OA has proven to be more complex than previously assumed. Prior to the beginning of this doctoral study, numerous chromosomal regions had been linked to OA in different populations and many genes important for cartilage homeostasis had been shown to harbor OA associated variants. To date, the general view of OA genetics with various linkage results and genetic associations still has not taken shape. It is, however, probable that once a sufficient amount of information on OA is provided through various independent genetic studies, we will be able to obtain a better understanding of the cause of OA as a whole and perhaps in the future develop new therapeutic interventions.

This study was thus undertaken in order to provide new information on the genetic background of this common disease. The specific aims were:

1. to study the role of the aggrecan gene (AGC1) VNTR polymorphism in hand osteoarthritis in a Finnish population,
2. to study the role of common promoter variations within the gene of interleukin 6 (IL6) in different forms of DIP OA,
3. to investigate the association of known SNPs and the VNTR polymorphism within the interleukin 1 gene cluster with DIP OA;
4. to perform a genome-wide linkage analysis in a sample of Finnish families manifesting primary osteoarthritis of the hip and/or knee in order to reveal chromosomal susceptibility loci and possible new candidate gene(s) for the disease.
4 Materials and methods

The materials and study populations used in this thesis are described in detail in original articles I–IV. Also, detailed descriptions of the common human genetic and statistical methods used in this study together with their references are presented in original articles I–IV and summarized in Table 4 below.

Table 4. Methods used in the present study.

<table>
<thead>
<tr>
<th>Method</th>
<th>Original publication</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Laboratory procedures</strong></td>
<td></td>
</tr>
<tr>
<td>DNA extraction</td>
<td>I, II, III, IV</td>
</tr>
<tr>
<td>Polymerase Chain Reaction (PCR)</td>
<td>I, II, III, IV</td>
</tr>
<tr>
<td>Gel electrophoresis</td>
<td>I, II, III, IV</td>
</tr>
<tr>
<td>Restriction enzyme analysis</td>
<td>I, III</td>
</tr>
<tr>
<td>Sequencing</td>
<td>II, III, IV</td>
</tr>
<tr>
<td>Southern hybridization</td>
<td>I</td>
</tr>
<tr>
<td><strong>Statistical Methods</strong></td>
<td></td>
</tr>
<tr>
<td>Fisher’s test</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Chi-square test</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Student’s t test</td>
<td>II</td>
</tr>
<tr>
<td>Haploview</td>
<td>II, III</td>
</tr>
<tr>
<td>PHASE</td>
<td>II, III</td>
</tr>
<tr>
<td>Pedcheck</td>
<td>IV</td>
</tr>
<tr>
<td>MLINK/LINKAGE</td>
<td>IV</td>
</tr>
<tr>
<td>Simwalk</td>
<td>IV</td>
</tr>
<tr>
<td>GeneMapper</td>
<td>IV</td>
</tr>
</tbody>
</table>

4.1 Study populations

Two independent populations were used in this study. Five hundred forty-three women aged 45 to 63 years with similar academic backgrounds (dentists and teachers) were recruited for studies I–III through the registers of the Finnish Dental Association and the Finnish Teachers Trade Union. Participating in a clinical examination were 295 (67.7%) dentists and 248 (56.9%) teachers. In addition to the clinical examination, all the participants were radiographed and evaluated for the presence and grade of OA in different hand joints. Disease outcomes for the AGC1 VNTR analysis (I) were based on the common disease patterns frequently observed among females with HOA (Solovieva et al. 2005). Because the OA of the DIP joints is a homogenous disease form and has yielded
positive results in genome scans compared to other OA phenotypes (Leppävuori et al. 1999, Hunter et al. 2004), it was chosen for a phenotype in the subsequent studies (II and III). The DIP joints were also the most commonly affected joints in this sample. Due to the assumption that inflammatory mediators are likely to associate with the symptoms of OA, the presence of symptoms was also taken into account in studies II and III. Bilateral OA was required to reduce the possibility of OA-specific changes occurring in a single joint by chance. Furthermore, due to the limited number of OA cases, only one pair of affected joints was required for the symmetrical OA definition in study II.

Another study sample (IV) consisted of 15 independent families with multiple subjects affected with primary OA of the hip and/or knee joint(s). All families originated from Central Finland and family members were evaluated for the presence of primary OA clinically and by radiologic imaging. Ten families with a total of 225 subjects were used for the genome-wide linkage analysis and an additional five families including 54 members were recruited for the following fine mapping and association analyses. A total of 279 individuals in 15 families were analyzed, consisting of 58 subjects with OA diagnosis of the hip and/or knee and 34 subjects that were considered healthy. The OA status of 185 subjects was considered unknown due to the strict exclusion criteria, including possible secondary causes and inflammatory joint diseases resembling OA.

4.2 Southern hybridization

The Southern hybridization technique used in original article I was performed as follows: five micrograms of genomic DNA from each sample was digested with HaeIII and fractionated on a 1.3% agarose gel for 20 h at 46V using a 100 bp DNA Step Ladder as a molecular weight marker. DNA on the gel was denatured (0.5 M sodium hydroxide, sodium chloride 1.5 M), neutralized (1.5 M sodium chloride, 0.5 M Tris - hydrochloric acid, 1 mM EDTA) and transferred to a nylon membrane using capillary action. The membrane was then exposed to ultraviolet radiation to permanently attach the transferred DNA and pre-washed for 1 h at 65 ºC in 0.1 X SSC (SSC, 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0), 0.5% SDS. The probe used in the analysis was a PCR amplified fragment from the VNTR area. The primer sequences used were provided previously by Doege et al. (1997). Labelling of the probe was performed with $[\alpha^{32}\text{P}]$dCTP using the Redprime II DNA Labelling system (Amersham Corp.). Prehybridization and hybridization were executed in hybridization buffer (SSC, 50% formamide, 10%
50-fold Denhardt's solution, 1 M sodium phosphate, 0.5% SDS, 10 mg/ml ssDNA) at a temperature of 42 °C for 2 h and 16 h, respectively. After hybridization, excess probe was washed from the membrane (2 x SSC, 0.1% SDS) in 37 °C and then 65 °C. The pattern of hybridization was visualized on X-ray film by autoradiography and the number of 57 bp repeats was then estimated from the film.

4.3 Linkage analysis

Genotyping for genome-wide screening was performed at the Finnish Genome Center (Helsinki, Finland) using the Applied Biosystems Linkage Mapping Set (MD 10) and an automated instrument (Megabase 1000; Molecular Dynamics). In the first fine mapping, an additional 10 markers and 7 markers (http://www.ncbi.nlm.nih.gov) spaced 2–5 cM apart were selected from the regions of interest on chromosomes 2 and 11, respectively. In the second fine mapping, 15 additional markers from chromosome 2 and 10 markers from chromosome 11 were genotyped to narrow down the space between markers to ~1 cM. Genetic distances (cM) and microsatellite marker locations were taken from the MAP-O-MAT sex-average linkage map (available at http://compgen.rutgers.edu/mapomat) and the database of the National Center for Biotechnology Information (NCBI). Standard PCR amplifications of the marker areas were performed according to the conditions described in detail in the article IV. The genotyping was performed using standard fluorescence-based genotyping methodologies (ABI PRISM 3100 Genetic Analyser, Applied Biosystems) and analyzed using GeneMapper Software (version 4.0, Applied Biosystems).

Both parametric and non-parametric methods were used in the study. Penetrance models were age-dependent (see Table 2 in article IV) and the disease allele frequency was set to 0.001. Parametric and non-parametric linkage analyses were performed in two-point and multipoint ways.

Calculation of the likelihood is complex due to the multiple markers and family members included in the study. Also, parental genotype data is often inadequate, with the result that all possible genotype and haplotype configurations must be taken into account. Furthermore, all possible disease locus genotypes need to be considered due to the incomplete penetrance. Thus, specific computer programs were used to estimate the linkage in different loci. In this study, both parametric and non-parametric multipoint LOD scores were calculated with Simwalk2 version 2.9 (Sobel & Lange 1996). Two-point LOD scores were...
obtained using the MLINK of the LINKAGE program package version 5.2 (Lathrop et al. 1984).
5 Results

5.1 Aggrecan VNTR polymorphism and hand osteoarthritis (I)

According to the radiographic measurements, three different definitions of outcomes were chosen for hand OA in this study: (i) the subject was defined to have OA if at least two of the finger joints were affected by OA of grade 2 (modified Kellgren and Lawrence system) or more, (ii) OA was moderately severe if at least one finger joint was affected by OA of grade 3 or more, and (iii) symmetrical OA if at least two pairs of joints were symmetrically affected by OA of grade 2 or more. The number of 57 bp repeats in the aggrecan VNTR locus was successfully detected by the Southern hybridization method from 530 subjects (dentists, n = 288 and teachers, n = 242). Among these subjects, the prevalence of OA, moderately severe OA and symmetrical OA were 42.6%, 14.7% and 19.9%, respectively.

5.1.1 VNTR allele frequency

Fourteen different alleles with 18, 21–30 and 32–34 repeats were identified (alleles A18, A21–A30 and A32–A34, respectively). Alleles A25–A29 represented 96.3% of the total allele count (n = 1060). The most common allele was A27 (38.6%) followed by A28 (30.7%) and A26 (16.8%). The total frequencies of the observed alleles are presented in Table 5 below. There were no differences between the studied occupational groups. Furthermore, individuals with and without radiographic findings showed no significant differences in terms of allele frequencies.

<table>
<thead>
<tr>
<th>Alleles</th>
<th>n</th>
<th>%</th>
<th>Alleles</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A18</td>
<td>1</td>
<td>0.1</td>
<td>A27</td>
<td>409</td>
<td>38.6</td>
</tr>
<tr>
<td>A21</td>
<td>6</td>
<td>0.6</td>
<td>A28</td>
<td>325</td>
<td>30.7</td>
</tr>
<tr>
<td>A22</td>
<td>8</td>
<td>0.7</td>
<td>A29</td>
<td>57</td>
<td>5.4</td>
</tr>
<tr>
<td>A23</td>
<td>2</td>
<td>0.2</td>
<td>A30</td>
<td>9</td>
<td>0.8</td>
</tr>
<tr>
<td>A24</td>
<td>2</td>
<td>0.2</td>
<td>A32</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>A25</td>
<td>51</td>
<td>4.8</td>
<td>A33</td>
<td>9</td>
<td>0.8</td>
</tr>
<tr>
<td>A26</td>
<td>178</td>
<td>16.8</td>
<td>A34</td>
<td>1</td>
<td>0.1</td>
</tr>
</tbody>
</table>
5.1.2 The most common aggrecan VNTR allele, A27, is protective against hand osteoarthritis

Ninety-five of the studied 530 women were homozygous for the most common A27 allele (the A27A27 genotype) and had a clearly reduced risk of OA compared to 216 non-carriers of the same allele with odds ratios (ORs) of 0.46 for OA, 0.40 for symmetrical OA and 0.42 for moderately severe OA. The corresponding 95% confidence intervals (CIs) were 0.27–0.78, 0.20–0.83 and 0.18–0.96, respectively.

Due to the low frequency of independent shorter alleles A18–A25 and the longer alleles A30–A34 (< 1%), these were combined in the statistical analysis. Women that carried two copies of the longer alleles (A28–A34, n = 93) had an increased risk of OA with an OR of 1.73 (95% CI 1.03–2.89). Furthermore, the risk of moderately severe OA was elevated among women carrying two copies of the shorter alleles (A18–A26, n = 50), but this result was not formally significant after adjustment for multiple testing. The ORs with corresponding CIs for the different disease outcomes are presented in Table 6.

Table 6. Association between the aggrecan VNTR alleles and radiographic findings.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Genotype</th>
<th>OA</th>
<th></th>
<th></th>
<th></th>
<th>Moderately severe OA</th>
<th></th>
<th></th>
<th></th>
<th>Symmetrical OA</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>OR</td>
<td>95%CI</td>
<td>N</td>
<td>OR</td>
<td>95%CI</td>
<td>N</td>
<td>OR</td>
<td>95%CI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A18–A26</td>
<td>Homozygous</td>
<td>25</td>
<td>1.30</td>
<td>0.69–2.42</td>
<td>13</td>
<td>2.45</td>
<td>1.17–5.12</td>
<td>13</td>
<td>1.45</td>
<td>0.71–2.96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heterozygous</td>
<td>60</td>
<td>0.93</td>
<td>0.62–1.41</td>
<td>24</td>
<td>1.37</td>
<td>0.78–2.40</td>
<td>30</td>
<td>1.12</td>
<td>0.68–1.86</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absence</td>
<td>139</td>
<td>1.00</td>
<td></td>
<td>41</td>
<td>1.00</td>
<td></td>
<td>62</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A27</td>
<td>Homozygous</td>
<td>28</td>
<td>0.46</td>
<td>0.27–0.78</td>
<td>8</td>
<td>0.42</td>
<td>0.18–0.96</td>
<td>11</td>
<td>0.40</td>
<td>0.20–0.83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heterozygous</td>
<td>83</td>
<td>0.85</td>
<td>0.57–1.29</td>
<td>28</td>
<td>0.82</td>
<td>0.48–1.43</td>
<td>37</td>
<td>0.78</td>
<td>0.47–1.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absence</td>
<td>102</td>
<td>1.00</td>
<td></td>
<td>38</td>
<td>1.00</td>
<td></td>
<td>52</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A28–A34</td>
<td>Homozygous</td>
<td>47</td>
<td>1.73</td>
<td>1.03–2.89</td>
<td>15</td>
<td>1.00</td>
<td>0.51–1.98</td>
<td>23</td>
<td>1.57</td>
<td>0.85–2.87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heterozygous</td>
<td>94</td>
<td>1.36</td>
<td>0.91–2.04</td>
<td>28</td>
<td>0.82</td>
<td>0.47–1.42</td>
<td>44</td>
<td>1.28</td>
<td>0.78–2.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absence</td>
<td>83</td>
<td>1.00</td>
<td></td>
<td>35</td>
<td>1.00</td>
<td></td>
<td>38</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Odds ratios (OR) and their 95% confidence intervals (CI) are adjusted for age and occupation. Subjects were defined as having OA if at least two of the finger joints were affected by OA of grade 2 or more, moderately severe OA if at least one finger joint was affected by OA of grade 3 or more, and symmetrical OA if at least two pairs of joints were symmetrically affected by OA of grade 2 or more. \( p_{\text{corr}} = 0.11; \)
\( p_{\text{corr}} = 0.012; \)
\( p_{\text{corr}} = 0.12; \)
\( p_{\text{corr}} = 0.039; \)
\( p_{\text{corr}} = 0.036. \)
5.2 Interleukin 6 promoter polymorphisms and symptomatic distal interphalangeal osteoarthritis (II)

In this study, DIP OA was defined by the presence of radiographic findings of grade 2 (modified KL system) in at least two DIP joints. Symmetrical DIP OA was defined by the presence of radiographic findings of grade 2 or more in at least one symmetrical pair of the DIP joints, and symptomatic DIP OA was defined by the same radiographic findings as DIP OA, accompanied by common symptoms, such as pain and tenderness in the affected joints. Three different sites with SNPs at promoter positions -597, -572 and -174 (G-597A, G-572C and G-174C) were investigated successfully from 535 participants, of whom 224 subjects were diagnosed as having DIP OA, 205 as having symmetrical DIP OA and 48 as having symptomatic OA. None of these conditions could be identified in 309 subjects.

All genotype frequencies were in Hardy-Weinberg equilibrium, and the two observed occupational groups showed no statistically significant differences in genotype frequencies or carriage rates. No significant association was observed between any of the studied SNP genotypes and DIP OA (radiographic OA).

5.2.1 G-alleles of the promoter polymorphisms associate with symptomatic distal interphalangeal osteoarthritis

A significant association was observed between symptomatic DIP OA and the G alleles at promoter positions -174 and -597, with the G allele being more common in subjects with symptomatic DIP OA (at -174, 57.3% compared to 43.4%, \( p_{\text{corrected}} = 0.01 \), and at -597 58.5% and 44.5%, \( p_{\text{corrected}} = 0.02 \)). Furthermore, 67.6% (n = 328) of the subjects without symptomatic DIP OA had at least one G allele at -174 versus 87.5% (n = 42) of the subjects with the disease. The corresponding difference for G allele carriage rates at -597 were 68.2% (n = 331) and 87.2% (n = 41), respectively (p = 0.007). At G-174C the combined GG and GC genotypes increased the risk of the disease in comparison with the CC genotype (p = 0.008). The same pattern was also observed with the G-597A polymorphism (p = 0.006). None of the genotypes at the G-572C polymorphic location had any effect on the risk of symptomatic DIP OA. No significant differences were seen in the genotypes or G allele frequencies between subjects with asymptomatic DIP OA or symmetrical DIP OA and subjects without these conditions.
5.2.2 Haplotype analysis of the promoter polymorphisms

A total of five haplotypes were observed of which the two most common ones were CGA (0.53) and GGG (0.40). The combined frequency of the other three haplotypes was only 0.07. No significant association was observed between a haplotype containing G allele at each locus and radiographic DIP OA or symmetrical DIP OA. Yet, the G-G-G haplotype was overrepresented in the subjects with symptomatic DIP OA in comparison with those without the disease (0.51 versus 0.39, p = 0.023). Furthermore, analysis of the haplotype pairs (diplotypes) showed that the G-G-G/other diplotype was also overrepresented among the subjects with symptomatic DIP OA (0.71 versus 0.46, p = 0.001). The risk of symptomatic DIP OA was also increased among the carriers of the G-G-G haplotype. Results of the haplotype analysis with ORs and corresponding confidence intervals (CI) are presented in Table 7 below.
Table 7. Frequency of the IL-6 G-G-G diplotypes according the DIP OA status (n = 533 to 535).

<table>
<thead>
<tr>
<th>Diploypes</th>
<th>No</th>
<th>Yes</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>DIP OA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>other/other</td>
<td>117</td>
<td>37.9</td>
<td>74</td>
</tr>
<tr>
<td>G-G-G/other</td>
<td>148</td>
<td>47.9</td>
<td>107</td>
</tr>
<tr>
<td>G-G-G/G-G-G</td>
<td>44</td>
<td>14.2</td>
<td>43</td>
</tr>
<tr>
<td>G-G-G carriage</td>
<td>192</td>
<td>62.1</td>
<td>150</td>
</tr>
<tr>
<td>G-G-G frequency</td>
<td>236</td>
<td>38.2</td>
<td>193</td>
</tr>
<tr>
<td>Symmetrical DIP OA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>other/other</td>
<td>127</td>
<td>38.6</td>
<td>64</td>
</tr>
<tr>
<td>G-G-G/other</td>
<td>155</td>
<td>47.1</td>
<td>101</td>
</tr>
<tr>
<td>G-G-G/G-G-G</td>
<td>47</td>
<td>14.3</td>
<td>40</td>
</tr>
<tr>
<td>G-G-G carriage</td>
<td>202</td>
<td>61.4</td>
<td>141</td>
</tr>
<tr>
<td>G-G-G frequency</td>
<td>229</td>
<td>35.9</td>
<td>181</td>
</tr>
<tr>
<td>Symptomatic DIP OA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>other/other</td>
<td>185</td>
<td>38.0</td>
<td>6</td>
</tr>
<tr>
<td>G-G-G/other</td>
<td>233</td>
<td>45.8</td>
<td>34</td>
</tr>
<tr>
<td>G-G-G/G-G-G</td>
<td>79</td>
<td>16.2</td>
<td>8</td>
</tr>
<tr>
<td>G-G-G carriage</td>
<td>302</td>
<td>62.0</td>
<td>42</td>
</tr>
<tr>
<td>G-G-G frequency</td>
<td>381</td>
<td>39.1</td>
<td>50</td>
</tr>
</tbody>
</table>

DIP OA was defined by the presence of radiographic findings of grade 2 or more in at least two DIP joints. Symmetrical DIP OA was defined by the presence of radiographic findings of grade 2 or more in at least one symmetrical pair of the DIP joints. Symptomatic DIP OA was defined by the presence of both radiographic findings of grade 2 or more and symptoms in at least two DIP joints.

*p-value remained statistically significant after correcting for multiple testing.

5.3 Interleukin 1 gene cluster polymorphisms and bilateral distal interphalangeal osteoarthritis (III)

In this study, the chosen disease outcome was bilateral DIP OA. If the subject had radiographic findings (grade ≥ 2) in at least one symmetrical pair of the DIP joints (if one DIP joint of the hand is affected, the same joint of the opposite hand is also affected), she was classified as having bilateral DIP OA. Among the 295 dentists and 248 teachers, the prevalence of bilateral DIP OA was 38% (46% in teachers and 31% in dentists). Within the interleukin 1 gene cluster, a total of nine SNPs were genotyped within the genes coding for the interleukin-1 type I receptor (IL1R1 on 2q12), interleukin-1 receptor-like 2 protein (IL1RL2 on 2q12),
interleukin-1α (IL1A on 2q13) and interleukin-1β (IL1B on 2q13). Also, a VNTR polymorphism in intron 2 of the IL1RN gene (IL1RN on 2q14.2) was genotyped resulting in the identification of four different alleles: allele 1 (four repeated units) had a frequency of 0.73, allele 2 (two repeats) a frequency of 0.25, allele 3 (three repeats) a frequency of 0.002, and allele 4 (five repeats) a frequency of 0.001.

To ensure laboratory quality control in genotyping tests, two independent readers interpreted the results. In every genotyping analysis, internal control samples with known genotypes were included. Any sample with ambiguous results was re-tested, and a random selection of 10% of all samples was repeated. No discrepancies were discovered upon replicate testing with the method used. For technical reasons, seven of the 543 samples could not be genotyped for the IL1R1, IL1RL2 and IL1RN polymorphisms.

5.3.1 Frequency of the alleles, genotypes and carriage rates of the studied polymorphisms

No statistically significant differences between the two studied occupational groups were seen in the frequency of alleles, genotypes or carriage rates of the studied polymorphisms. Nevertheless, a statistically significant association between one SNP in \textit{IL1B} (rs1143633) and bilateral DIP OA was obtained with OR of 3.03 (95% CI 1.35-6.83, \( p = 0.006 \)) under the recessive model of inheritance. A significant association between the SNPs in \textit{IL1R1}, \textit{IL1RL2}, \textit{IL1A} and \textit{IL1B} (rs16944) and between the \textit{IL1RN} VNTR polymorphism and bilateral DIP OA could not be seen. Results of the association analysis between all studied polymorphisms and bilateral DIP OA are presented in Table 8. A considerable linkage equilibrium (LD) occurred between the studied SNPs in the \textit{IL1R1} and \textit{IL1RL2} genes and between the \textit{IL1RN} VNTR polymorphism and either \textit{IL1A} or \textit{IL1B} SNPs. (See the LD plot in original article III).
Table 8. Association of the *IL1* gene cluster polymorphisms with bilateral DIP OA.

<table>
<thead>
<tr>
<th>SNPs ID</th>
<th>Gene</th>
<th>Geno-type</th>
<th>Counts</th>
<th>OR²</th>
<th>95% CI</th>
<th>p-value¹</th>
<th>p</th>
<th>D</th>
<th>A</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1465325</td>
<td>T/T</td>
<td>204/124</td>
<td>1</td>
<td>0.42</td>
<td>0.39</td>
<td>0.25</td>
<td>0.24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IL1R1</em></td>
<td>T/C</td>
<td>115/72</td>
<td>1.13</td>
<td>0.76–1.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>9/10</td>
<td>1.86</td>
<td>0.70–4.93</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs956730</td>
<td>G/G</td>
<td>164/94</td>
<td>1</td>
<td>0.51</td>
<td>0.30</td>
<td>0.47</td>
<td>0.82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IL1R1</em></td>
<td>G/A</td>
<td>138/97</td>
<td>1.25</td>
<td>0.85–1.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>26/15</td>
<td>1.02</td>
<td>0.50–2.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2287047</td>
<td>C/C</td>
<td>158/103</td>
<td>1</td>
<td>0.79</td>
<td>0.49</td>
<td>0.51</td>
<td>0.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IL1R1</em></td>
<td>C/T</td>
<td>135/87</td>
<td>1.13</td>
<td>0.77–1.67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>25/16</td>
<td>1.17</td>
<td>0.57–2.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1922290</td>
<td>G/G</td>
<td>138/82</td>
<td>1</td>
<td>0.42</td>
<td>0.53</td>
<td>0.99</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IL1RL2</em></td>
<td>G/C</td>
<td>128/90</td>
<td>1.24</td>
<td>0.82–1.86</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>62/34</td>
<td>0.90</td>
<td>0.53–1.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1922295</td>
<td>G/G</td>
<td>137/82</td>
<td>1</td>
<td>0.45</td>
<td>0.56</td>
<td>0.99</td>
<td>0.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IL1RL2</em></td>
<td>G/A</td>
<td>145/98</td>
<td>1.20</td>
<td>0.81–1.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>46/26</td>
<td>0.87</td>
<td>0.48–1.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1800587</td>
<td>C/C</td>
<td>149/91</td>
<td>1</td>
<td>0.63</td>
<td>0.59</td>
<td>0.90</td>
<td>0.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IL1A</em></td>
<td>C/T</td>
<td>145/95</td>
<td>1.16</td>
<td>0.79–1.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>41/21</td>
<td>0.90</td>
<td>0.48–1.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1143634</td>
<td>C/C</td>
<td>189/100</td>
<td>1</td>
<td>0.06</td>
<td>0.02</td>
<td>0.02</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IL1B</em></td>
<td>C/T</td>
<td>123/88</td>
<td>1.53</td>
<td>1.03–2.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>23/19</td>
<td>1.76</td>
<td>0.87–3.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1143633</td>
<td>G/G</td>
<td>170/118</td>
<td>1</td>
<td>0.001</td>
<td>0.13</td>
<td>0.88</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IL1B</em></td>
<td>G/A</td>
<td>154/69</td>
<td>0.62</td>
<td>0.42–0.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>11/20</td>
<td>2.48</td>
<td>1.08–5.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs16944</td>
<td>C/C</td>
<td>121/85</td>
<td>1</td>
<td>0.48</td>
<td>0.24</td>
<td>0.37</td>
<td>0.91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IL1B</em></td>
<td>C/T</td>
<td>167/92</td>
<td>0.78</td>
<td>0.52–1.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>47/30</td>
<td>0.85</td>
<td>0.48–1.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IL1RN</em></td>
<td>1/1</td>
<td>185/114</td>
<td>1.00</td>
<td>0.14</td>
<td>0.86</td>
<td>0.54</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VNTR</td>
<td>1/2</td>
<td>110/77</td>
<td>1.20</td>
<td>0.80–1.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/2</td>
<td>35/14</td>
<td>0.58</td>
<td>0.28–1.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Uncorrected p-value of the association analysis; D = Dominant; A = Additive; R = Recessive. ²ORs and 95% CIs are adjusted for age, occupation, BMI and smoking history. For the *IL1RN* variable-number tandem repeat (VNTR) polymorphism, allele 1 consists of the alleles with 4 repeat units, 3 repeat units and 5 repeat units; allele 2 has 2 repeat units.
5.3.2 Analysis of the extended haplotypes

Separate haplotype analyses for the IL1R1-IL1RL2 and IL1B-IL1RN SNPs were conducted due to the weak LD between the two regions. Seven different haplotypes were analyzed and no difference was observed between subjects with and without bilateral DIP OA in the IL1R1-IL1RL2 haplotype distribution. Yet, the IL1R1-IL1RL2 112 haplotype was associated with an increased risk of OA among the dentists (OR = 1.75, 95% CI 1.15–2.68, p = 0.009) and a reduced risk of OA among the teachers (OR = 0.66, 95% CI 0.43–1.01, p = 0.05).

It was hypothesized that among the dentists who are exposed to continuous repetitive joint usage, the haplotype might modify this environmental factor. Thus, the possible interaction between the haplotype and occupational hand load among the dentists was also examined. It seemed that the haplotype exacerbates the effect of low variation in dental work tasks on the risk of OA, but there was no effect of the haplotype in dentists with high variation in work tasks (dentists with low variation in work tasks and the carriers of the haplotype: OR = 2.25, 95% CI 0.91–5.54, p = 0.08, and dentists with high variation in work tasks and non-carriers of the haplotype: OR = 0.73, 95% CI 0.28–2.01, p = 0.54).

A total of 16 different IL1B-IL1RN VNTR haplotypes were reconstructed and in general, none of the haplotype allele frequencies differed significantly between subjects with and without DIP OA. Due to the low LD between the rs180057 (IL1A) and other investigated polymorphisms, four polymorphisms within the IL1B-IL1RN region were used to group the haplotypes for further analysis. The distribution of the 211-1 diplotype fitted an additive mode of inheritance, while the distribution of the 121-1 diplotype fitted a recessive mode (OR = 1.42, 95% CI 1.05–1.94, p = 0.025 and OR = 4.36, 95% CI 1.48–12.83, p = 0.007, respectively). A difference between the two occupational groups was also seen in extended haplotypes constructed from IL1B-IL1RN. The 211-1 haplotype was associated with an increased risk of OA among the dentists only with OR 2.39, 95% CI 1.30–4.41, p = 0.005. In addition, the 111-1 haplotype was associated with a reduced risk of OA among the teachers (OR = 0.43, 95% CI 0.20–0.93, p = 0.03) and an increased risk among the dentists (OR = 2.23, 95% CI 1.04–4.80, p = 0.04).

Concerning the amount of dental tasks, both low variation in work tasks and the carriage of the 211-1 haplotype had an effect on the risk of OA (OR = 3.45, 95% CI 1.22–9.74, p = 0.02 and OR = 5.69, 95% CI 1.86–17.42, p = 0.002, respectively). However, the combined effect of these two factors on the risk of
OA was similar to the effect of the haplotype on the disease risk (OR = 5.63, 95% 1.93–16.46, p = 0.002).

5.4 Linkage analysis of primary hip and knee osteoarthritis (IV)

In this study, the diagnosis of hip and knee OA was based on the following criteria: Subjects were required to have suffered chronically from common OA symptoms in the affected hip or knee joint(s) and typical radiological findings associated with OA were to be seen in the standard x-ray joint projections. Subjects that were known to have any secondary cause of OA or symptoms of any other confounding rheumatoid joint disease were excluded from the study.

In this sample of a total of fifteen families, the average age of the disease onset was 50 years, although in many cases the first symptoms of joint disease had been noticed before the age of 40. Among the 279 studied individuals, OA of the hip joint was present in 23 subjects, OA of the knee joint in 20 subjects and 15 subjects were affected with both conditions. Characteristics of the studied families by the OA status are presented in Table 9.

Table 9. Characteristics of the studied families with the definitions of OA outcomes used for the linkage analysis.

<table>
<thead>
<tr>
<th>Family</th>
<th>n</th>
<th>Male</th>
<th>Female</th>
<th>Hip OA</th>
<th>Knee OA</th>
<th>Knee &amp; Hip OA</th>
<th>Healthy</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>4</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>14</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>13</td>
<td>18</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>6</td>
<td>8</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>37</td>
<td>16</td>
<td>21</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>33</td>
<td>17</td>
<td>16</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>All</td>
<td>279</td>
<td>137</td>
<td>142</td>
<td>23</td>
<td>20</td>
<td>15</td>
<td>34</td>
<td>185</td>
</tr>
</tbody>
</table>

1 Three affected individuals were identified in family 1, but DNA was available only from one.
5.4.1 Genome-wide scan

The whole genome scan of 225 individuals in ten different families resulted in the identification of four interesting loci on chromosomes 2, 11, 13 and 20 with logarithm of odds (LOD) scores above 1.5 using two-point linkage analysis. The same region on chromosome 2 (between markers D2S112 and D2S142) resulted in a LOD score of 2.14 in multipoint analysis, which was the highest peak detected in the genome wide scan. The second highest peak was detected on chromosome 11 with a maximum multipoint LOD score of 1.82.

5.4.2 Fine mapping

The two loci with the highest LOD scores at Chr 2 and 11 were further analyzed by fine mapping. Five additional families were recruited with 54 individuals. The linkage signal for Chr 11 disappeared in the fine mapping. Nevertheless, the susceptibility of Chr 2 was confirmed with a multipoint LOD score of 3.91 under the recessive model of inheritance. The same, approximately 7 cM region at 2q21 was also positive in two-point linkage analysis with a LOD score of 3.63 (see Figure 7 below). Surprisingly, mainly one family (family 10) contributed to the positive LOD scores in all analyses. This family consisted of a total of 33 studied members. The gender distribution was 17 males and 16 females from three generations, of whom primary OA of the hip joint was identified in 5 family members, OA of the knee joint in 1 family member and both conditions in 4 family members. Only 6 family members could be certainly defined as healthy concerning OA. Among 17 family members, the presence/absence of OA could not be confirmed and the status of these individuals was therefore considered unknown in the analyses. The pedigree of this family is presented in Figure 8.
5.4.3 Association analyses of potential candidate genes

Several variations within the candidate genes at the positive linkage loci were analyzed. Coding regions and intron boundaries of TSG6, NURR1, CXCR4, FRZB, ACVR1 and CFC1 were analyzed and several single nucleotide polymorphisms in the IL1A, IL1B, IL1R1 and IL1RL2 genes were genotyped. There was no statistically significant difference in the genotype frequencies between the affected and healthy family members in any of the analyzed variations.
Fig. 8. Pedigree of family 10. OA affected individuals are indicated by black circles and unaffected by white. Circles represent females and squares, males. Individuals marked with a diagonal line across the symbol are deceased. Status of OA could not be determined from individuals marked with a question mark.
6 Discussion

6.1 The complex genetics of osteoarthritis

OA is a very common disease resulting in the destruction of synovial joints. Many of the mechanisms involved in the disease cascade of OA have been identified, and important molecular and signaling mechanisms behind its pathology have been described. Furthermore, it is known that there are a number of environmental factors predisposing to the disease and that it is more prevalent among women. It can also be stated that the contribution of genetic factors to this condition is complex, yet undisputable. Despite all this knowledge, the treatment of OA is still primarily based on a desperate fight against the progressing symptoms that arise from the continuous slow destruction of articular cartilage. The onset of the disease cannot be prevented because we have not been able to find or recognize the underlying cause that initiates this process. Nevertheless, scientists studying the etiology of OA are unanimous that the answer to this problem, at least to some extent, lies in the genome.

Since the discovery of the familial accumulation of nodes, we have come a long way in learning the genetic etiology of OA. An important basis for the current knowledge has been the identification of multiple Mendelian disorders and syndromes characterized by the juvenile arthritic phenotype. It has also been shown that the genetics of these chondrodysplasias often involve genes of important structural proteins of cartilage (for a review, see Kannu et al. 2009). Nearly all of the 22 autosomal chromosomes have been reported to putatively harbor one or more loci that are transmitted from parent to offspring together with OA more often than expected under independent inheritance. Only a few of these regions showing linkage to OA overlap or are in close proximity with another linked region. Also, only a few of the reported loci have shown significant evidence for linkage. In addition to all of the susceptible loci, there are dozens of individual genes that are considered to harbor OA-related variants. Again, only a handful of the reported susceptibility variants have provided significant evidence for association and have been successfully replicated in independent populations/study cohorts. This raises a question: Are some of the reported linkage and association findings false positives, or is it possible that the genetic etiology of OA would be this complicated? It is likely that both of these alternatives are at least partly true.
The search for susceptibility genes has progressed during the last few years. At least to some extent, it might be because OA is now assumed to be not a single entity, but more likely a group of distinct diseases. This means that the contribution of genes may differ between sexes and according to the localization and pattern of the affected joints. This doctoral study suggests that even the development of symptoms in this disease may, at least partly, arise from a genetic variation. Applying some of the preceding stratifications to carefully selected patient material has generally led to better results in OA genetics. It is noteworthy that even though some of the candidate genes, for example \textit{ASPN}, are repeatedly shown to associate with OA, many times the results have not been successfully confirmed in other ethnic groups (Rodriguez-Lopez \textit{et al.} 2006). Generally, this leads to the assumption that the genetic etiology of OA may be significantly different among populations with varying ethnic backgrounds. This hypothesis is supported by the fact that there seems to be also small groups, even independent families that demonstrate the typical OA phenotype with unique predisposing genetic factors that cannot be identified in larger populations of the same origin, as discussed later in section 6.4. Another likely reason for the discrepancies seen in the results between different populations lies in the low statistical power of the study material, which is still common in OA studies.

The preceding points make it difficult to give a comprehensive description of the genetic etiology of OA because the results obtained to date are divergent. However, the recent success in GWA studies has begun to reveal common OA-associated variations that seem to be modest but universal. Despite the wealth of genetic data obtained from OA, a lot of the needed information is yet to be found. The significance and contribution of the work presented in this thesis on uncovering of the mystery of OA genetics is discussed in detail in the following sections.

6.2 Characteristics of the study populations

The study sample consisting of dentists and teachers was well characterized and large enough to show that the reported associations are plausible. Possible population stratification was avoided by random selection of subjects from a common geographical origin. However, defining the phenotype of OA in multiple ways significantly reduced the statistical power in these analyses and increased the possibility of a type 1 error. Thus, a larger sample size would have been beneficial. The study setting was cross-sectional, meaning that the relationship
between symptoms of OA, radiological findings and different genotypes were evaluated at a certain point in time. Therefore, one must take into account the possibility that the information collected concerning the symptoms of OA might have been different if the study setting was longitudinal. Utilizing two different occupational groups enabled the investigation of the relationship between different patterns of joint usage and analyzed genotypes. This was a clear strength of the study. Analyzed genetic variations were chosen based on their previously reported associations or known functionality. This increases the value of the reported results because, if valid, they may be useful in the estimation of the proportional risk of OA, as suggested previously (Valdes et al. 2009).

Families used in the linkage analysis had a common geographical origin. Only symptomatic OA was studied, which may have affected the results. Sizes of the families were generally adequate, but the number of affected individuals was relatively low, which is likely the reason why only one family was shown to contribute significantly to the resulting linkage. This matter is discussed in more detail in section 6.5. In light of recent studies, combining the hip and knee OA cases in the analysis could also be criticized. Unfortunately, the statistical power of this sample would have been inadequate for separate analysis.

### 6.3 Is the effect of the aggrecan VNTR polymorphism significant in the development of osteoarthritis? (I)

Aggrecan is the most abundant structural proteoglycan in articular cartilage and its degradation is among the first identified molecular events that take place in the beginning of OA (Magdalou et al. 2008). The common VNTR polymorphism in AGC1 has a clear biological function as the number of repeat sequences in the VNTR area correlate to the amount of GAG side chain binding sites in the mature protein. Prior to this study, only two previous association studies concerning the VNTR polymorphism and OA had been conducted with substantially smaller sample sizes and somewhat ambiguous results. Horton et al. (1998) reported that in a sample of 93 Caucasian men, allele A27 with 27 repeats increased risk of hand and knee OA (OR 3.23, CI 1.24–8.41), whereas Kirk et al. (2003) reported the deleterious effect of A27 and protective effect of alleles A25 and A28 among 134 twins for OA of the hand, hip and knee. In contrast to these previous findings, the results reported in the current study suggest that A27 is protective against HOA if the subject is homozygous for it. With the allele frequencies reported in this study (0.4 for OA cases and 0.53 for controls), the sufficient amount of cases
and controls in order to detect the association with 80% certainty were 210 and 294, respectively. Thus, the sample size in this study (224 affected and 306 controls with A27) was sufficient in terms of statistical power. A27 was also the most common allele in our sample. Furthermore, it was observed that carrying two copies of any of the shorter numbers of repeat sequences than 27 predisposes to more serious forms of HOA. Also, subjects with two copies of any longer alleles than 27 repeats had a slightly increased risk for severe HOA, but the low number of the alleles reduced the statistical power concerning this finding. The first two observations are supported by the biological function of the polymorphism. As A27 was the most common allele in our sample, it is logical to think that the amount of GAG attachment sites it encodes is sufficient for the functioning of the mature protein, and thus associates with lower disease frequency. Furthermore, the HOA predisposing effect of two shorter alleles could arise from the resulting inadequate amount of GAG attachment sites in the core protein that leads to reduced osmotic swelling pressure of the proteoglycan network during physical strain of the tissue. Another explanation of this finding could be the easier diffusion of partially cleaved aggrecan molecules, encoded by shorter alleles, into the synovial fluid as suggested in a recent study describing a similar association of the shorter alleles with RA (de Souza et al. 2008)

Despite the findings presented in this study and the preceding conclusions that can be drawn based on the known effect of the VNTR polymorphism on the molecular structure of aggrecan, it still remains unclear whether or not there is a direct link between aggrecan VNTR and OA. The number of reported associations so far is not convincing and needs to be confirmed in other populations that are large enough to demonstrate the possible predisposing effect of the rare alleles with either a very low or high repeat number. The low frequency of certain alleles may be another reason why the significance of the AGC1 gene in cartilage breakdown has been difficult to confirm. As discussed previously, the number of subjects with two rare alleles may be only one or two in a sample of hundreds of individuals. Yet, if at risk for OA, these subjects would represent a substantial proportion in the entire population (Roughley et al. 2006b). It is also a possibility that the AGC1 VNTR polymorphism is not directly linked to OA susceptibility, but is in linkage equilibrium with some other nearby susceptibility locus that has not yet been identified.

Even if the role of this VNTR polymorphism in the etiology of OA can be confirmed in the genetic studies undertaken in the future, a set of functional
studies are still needed in order to test the effect of different polymorphic alleles on the actual homeostasis of the proteoglycan network in the ECM.

6.4 Genetic factors affect the inflammation reaction observed in osteoarthritis cartilage (II, III)

IL-6 is an essential cytokine of the inflammation cascade. In OA cartilage, the level of IL-6 mRNA has proven to be elevated along with the amount of this cytokine in serum and synovial fluid in OA patients (Kaneko et al. 2000). Recently it was also reported that the expression of IL-6 is increased in the subchondral bone osteoblasts of OA tissue (Sakao et al. 2008) suggesting it is affecting cartilage metabolism during the disease. Furthermore, mechanical compression seems to increase the IL-6 production by osteoblasts (Sanchez et al. 2008). Among the various polymorphisms recognized in the IL-6 gene, those in the promoter area in particular have been reported to affect the transcriptional activity of the gene (Osiri et al. 1999, Jordanies et al. 2000, Ota et al. 2001). In the present study, it was observed that the G alleles at promoter positions -174 and -597 individually and also together were more common among subjects with symptomatic DIP OA. The predisposing effect of the G alleles was also evident when haplotypes constructed from variants G-597A, G-572C and G-174C were compared between subjects with or without symptomatic DIP OA. The haplotype G-G-G was overrepresented in the women with symptomatic DIP OA in comparison with those without the disease. Several previous and recent findings support these results and their possible contribution to the inflammation reaction present in OA: G alleles at -597 and -174 are related to increased ex vivo production of IL-6 (Rivera-Chavez et al. 2003), and at -174 individually, the G allele is related to increased expression and plasma levels of the molecule (Bennermo et al. 2004). The CC genotype at -174 was significantly higher among the control subjects in a hip OA population (Pola et al. 2005), and the G alleles in these three loci generally associate with increased transcription of IL-6 (Terry et al. 2000). The number of affected individuals in this study was rather low (n = 48, 9%). Furthermore, the only other reported association study by Pola et al. (2005) that showed OR 0.4 (CI 0.1–0.9, p = 0.04) for the carriage of the CC genotype had an even smaller sample size (75 patients and 107 controls). Thus, the reported associations need to be replicated in a larger OA sample. No associations between the studied genotypes or haplotypes and DIP OA without symptoms (ROA) were seen, which suggests that the studied cytokine may affect the development of the
symptoms through inflammatory pathways. Pain is a common feature of the inflammation reaction; its connection to IL6 is evident and has been observed concerning genotype GG at -174 also in patients with juvenile RA (Oen et al. 2005).

Taken together, these observations provoke the idea that the influence of IL-6 on the synovial joint could be therapeutically modified in order to treat OA. One such intervention could be a specific receptor antagonist that binds to the IL-6 receptor, preventing the response in target cells. However, designing a molecule with the desired properties could be difficult due to the fact that many cytokines share the same signaling pathway as IL-6, with some of the same structural receptor elements (Heinrich et al. 1998). Concerning IL-1, such an antagonist, named anakinra, is already in use to treat RA (Bingham 2008).

When studying the IL-1 gene cluster, it was observed in this sample that carrying of the minor alleles of two IL1B polymorphisms (rs1143633 and rs1143634) and the IL1B-IL1RN extended haplotypes (211-1 and 121-1) were associated with an increased risk of bilateral DIP OA. Also, extended haplotypes of IL1R1-IL1RL2 and IL1B-IL1RN associated with the disease, although, differently between the two occupational groups. It is possible that this kind of controversy within the cohort is caused by chance due to the multiple haplotype alleles analyzed in a study sample of limited size.

Numerous association studies have suggested the role of different SNPs and haplotypes contributing to the etiology of OA as stated in section 2.4.1. Functional studies have suggested that the combination of alleles may be an important aspect in the regulation of IL1 gene expression. The inflammatory response is regulated by a balance between pro- and anti-inflammatory cytokines. A risk haplotype reported here includes the alleles that can affect the production of both IL-1Ra and IL-1β (Hurme & Santila 1998, Vamvakopoulos et al. 2002). A certain combination of haplotypes could, therefore, result in the imbalance in cytokine production and contribute to the destructive process of cartilage (Martel-Pelletier et al. 1999). Subjects representing two different academic groups in terms of repetitive joint usage were analyzed in this study. It is known that the mechanical environment can have a substantial effect on cartilage homeostasis and on the production of inflammatory mediators. Stronger associations between the IL-1 gene cluster polymorphisms and OA observed among the dentists in this sample supports the idea that environmental factors can modulate the effect of modest genetic predisposing alleles on OA risk. Yet, due to the high LD rates observed in the area, the possibility remains that none of the reported IL-1 gene
cluster alleles are independently contributing to OA risk, but are in linkage
equilibrium with unidentified risk alleles situated nearby. In summary, it seems
rather certain that this group of genes is somehow contributing to the etiology of
OA, but further investigation is needed in order to clarify the role of the numerous
polymorphisms and their possible interaction in different forms of OA.

Despite the fact that the *IL-1* gene cluster and *IL-6* can be shown to play a
role in OA, one must acknowledge that there are also multiple other cytokines
present in OA cartilage and that the mechanisms of their interrelated regulation as
well as hierarchy are complex. Therefore, more information is needed before the
role of these molecules can be accurately defined in the disease process. It may
also be speculated that the recognized inflammation process in OA is actually a
secondary phenomenon, the consequence of the progressing degradation of
cartilage that has been originally initiated by totally different molecular events.

Nevertheless, if the role of the cytokines will prove to be significant in the
etiology of OA, modifying their effects in AC is likely to result in the
development of new therapeutic interventions.

### 6.5 Linkage analysis, a way to identify new osteoarthritis-
associated chromosomal loci (IV)

In this study, a new chromosomal locus that is in linkage with the primary OA of
hip and knee was identified in Chr 2q21 with a LOD score of 3.91. Chr 2 has
previously been shown to contain multiple loci that are in linkage with nodal OA
(Wright *et al.* 1996), HOA (Leppävuori *et al.* 1999, Demissie *et al.* 2002,
*et al.* 2002b) and generalized OA (Meulenbelt *et al.* 2006). Nevertheless, the
locus reported here does not overlap with any of the previously reported loci. In
the current study, the identification of a specific predisposing variation within the
linked region has been unsuccessful and thus, these results at this point mainly
strengthen the hypothesis that Chr 2 harbors multiple genetic variants that are
likely to be important in the disease etiology. Mainly, only one of the studied
families contributed significantly to the observed LOD. It may therefore be that
the underlying genetic variation is restricted to this family and does not
necessarily contribute to disease prevalence in others. These kinds of independent
families with unique disease-associated variations have been reported previously,
and the clinical outcome of the disease in these cases can be very similar to or
even indistinguishable from OA (Meulenbelt *et al.* 1997, Ingvarsson *et al.* 2001,
Jakkula et al. 2005, Mabuchi et al. 2006). It may be due to the limited number of affected individuals, however, which resulted in none of the other families contributing strongly to the observed LOD. It is also noteworthy that no significant linkage was observed using the dominant inheritance model in the analyses. Concerning complex diseases, it has been thought that the disease is more likely inherited according to the dominant Mendelian model. Nevertheless, this study suggests it is possible that some of the genetic features predisposing to the disease are transmitted from parent to offspring following the recessive model of inheritance. This assumption is also supported by the results obtained in the \textit{IL1} gene cluster association analysis (III).
7 Conclusions

Several genome-wide scans and an even greater number of association reports of individual genes conducted to date represent important milestones on the way towards better understanding of OA. So far, none of these milestones alone can be considered major enough to offer a convincing explanation of the disease etiology. If anything, individual results are pieces in a puzzle that is proving to be much larger than anticipated by former investigators of OA genetics. Many promising individual discoveries have been made since the beginning of this doctoral study; nevertheless, we are still unable to see what the puzzle illustrates.

A key to the mystery of OA genetics has traditionally been sought from the genes that are expected to be important for cartilage homeostasis. Mostly, this has not been as rewarding as one would expect. Variants within some of the genes of structural proteins clearly harbor OA susceptibility, but cannot be considered as major disease-causing factors. Recently, the focus of investigation has been more on the genes of different signaling molecules found in cartilage. Convincing results obtained with ASPN, FRZB, GDF5 and MATN3 prove that it is rather the signal transduction pathway, which is likely to be a major component in OA susceptibility. This is an important discovery, since these pathways are potentially modifiable and can therefore be suitable targets for therapeutic interventions. The question is: Will any of the known susceptibility loci prove to be compelling enough to provoke the development of a new treatment?

There is already a wealth of genetic data on OA that keeps building up as new susceptibility loci are reported more frequently than before. The knowledge of the molecular genetics is developing quickly, and we are already taking advantage of new powerful genotyping techniques that are likely to revolutionize the understanding of OA genetics in the near future. It is possible that as the genetics of OA will sharpen, the genes considered important in this study will be replaced by others that prove to harbor variations that are more essential for the disease etiology as a whole. Still, every locus that has proven to affect the susceptibility of OA may be important. The information from different association analyses could be gathered together in order to design a gene test to evaluate the individual risk of OA. Thus, a person carrying multiple genetic risk factors could avoid the known environmental factors that further predispose to the disease. This information could also be used to genotype chondrocytes that are used for autologous transplantation treatment in order to choose those cells with minimal OA predisposing loci.
This doctoral study underlines the importance of the careful selection of populations for future studies. Ethnically homogenous subjects with a common clinical outcome in terms of joint location and symptoms as well as strict exclusion of other possible confounding factors will help to ensure that the investigated genetic contribution, which in most cases has proven to be modest, will not be overwhelmed. It would also be beneficial if the same definitions and classifications of OA could be used among different populations. It is likely that the standards concerning these definitions of disease outcomes will develop in the future as our understanding of OA grows.

It remains to be seen whether we will continue to collect the small pieces of the puzzle or eventually find a major OA locus. The growing evidence of the complexity of this disease makes the latter option seem rather unlikely. Nevertheless, by gaining a little amount of new information step by step, we are on the way towards understanding why cartilage ultimately breaks down. It is a fundamental matter to solve if we are determined to overcome the burden of osteoarthritis.
References


Hurme M & Santtila S (1998) IL-1 receptor antagonist (IL-1Ra) plasma levels are coordinately regulated by both IL-1Ra and IL-1beta genes. Eur J Immunol 28: 2598–2602.


Sadouk MB, Pelletier JP, Tardif G, Kiansa K, Cloutier JM & Martel-Pelletier J (1995) Human synovial fibroblasts coexpress IL-1 receptor type I and type II mRNA. The increased level of the IL-1 receptor in osteoarthritic cells is related to an increased level of the type I receptor. Lab Invest 73(3): 347–55.


Original articles

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


Reprinted with permission from Elsevier (I) and The Journal of Rheumatology Publishing Company Limited (III).

Original publications are not included in the electronic version of the dissertation.


1005. Imataniemi, Sari (2009) Fall accidents and exercise among a very old home-dwelling population

1006. Westerlund, Tarja (2009) Thermal, circulatory, and neuromuscular responses to whole-body cryotherapy


1008. Kuisma, Mari (2009) Magnetic resonance imaging of lumbar degenerative bone marrow (Modic) changes. Determinants, natural course and association with low back pain


1010. Löfgren, Johan (2009) Genetic polymorphisms in collectins and Toll-like receptor 4 as factors influencing susceptibility to severe RSV infections and otitis media


1013. Tetri, Sami (2009) Factors affecting outcome after primary intracerebral hemorrhage


Olli-Pekka Kämäräinen

THE SEARCH FOR SUSCEPTIBILITY GENES IN OSTEOARTHRITIS