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## **Genes and mediators of inflammation and development in osteoarthritis**

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# 1

CC / CT / TT

The image is a composite of three main visual elements: a DNA double helix on the left side, a microscopic view of cells in the center, and a human pelvic X-ray on the right side. The background is a light gray with a repeating pattern of DNA base pairs (A, T, C, G) and the text 'CC / CT / TT'.

# General Introduction



## 1.1 General introduction

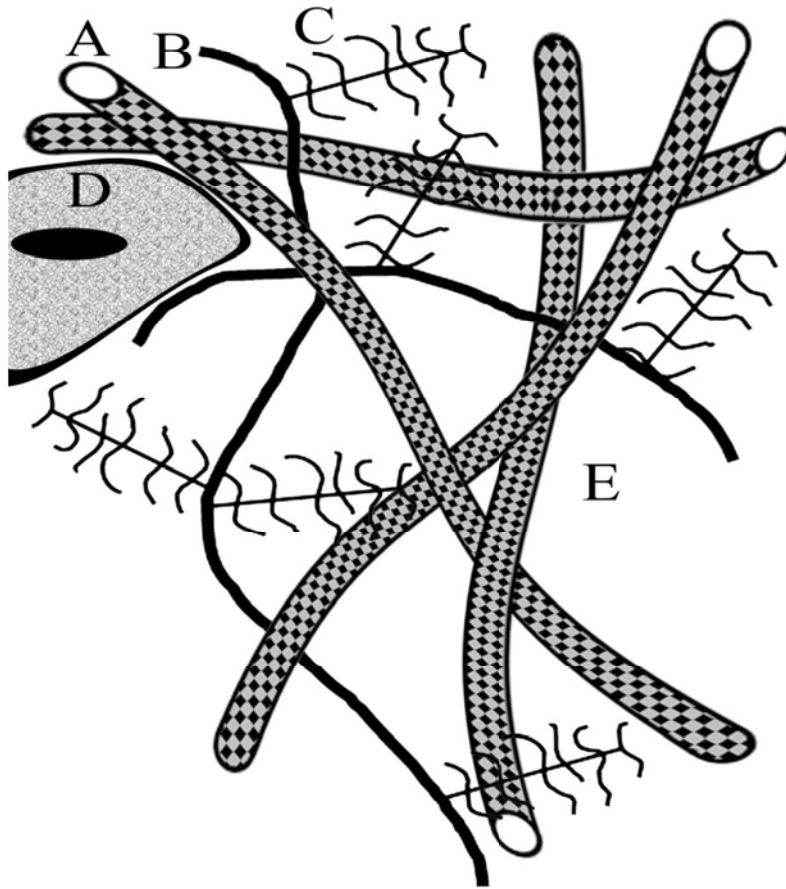
The term osteoarthritis (OA) is used to describe a heterogeneous group of common, age related musculoskeletal disorders, characterized by joint pain and limitations in joint laxity<sup>1</sup>. The main affected tissue in OA is the cartilage covering articular bones in joints, however, the disease affects all compartments of these articular joints. In addition to focal loss of cartilage, subchondral bone, ligaments and synovium show signs of involvement in OA<sup>1,2</sup>. Reliable diagnosis and classification of osteoarthritis by a comprehensive set of radiographic criteria was facilitated in 1957 by Kellgren and Lawrence<sup>2</sup>, which were illustrated in the “Atlas of standard radiographs of arthritis” in 1963<sup>3</sup>. To date this grading system is typically used by researchers for OA classification. The grading system is based on the decrease of articular cartilage thickness reflected by joint space narrowing and bone remodeling reflected by subchondral sclerosis and osteophyte formation. This score is implemented in an ordinal scaling system using 5 categories representing no OA signs (score 0) to severe OA (score 4). Radiographs are noninvasive and easily obtainable, however, for OA research the collection and reading of the radiographs is relatively expensive and time consuming as compared to collection through clinical assessment or identification of subjects meeting the clinical end point of OA by receiving joint replacements. Furthermore, research on progression of OA using radiographs is subject to high heterogeneity as a result of measurement methods and reading variability<sup>4,5</sup>.

Clinically, the OA diagnosis relies largely on manifestations of the disease such as pain and joint deformations. Between the clinical and radiological classification of OA a fair amount of disease heterogeneity is illustrated by poor correlation of these measures. The relation of the disease to age is demonstrated by a study performed in inhabitants of a Rotterdam area where going from mean ages of 55 to 70 years the prevalence of radiographic signs OA was increasing from 80% to 95% for any of the four major joint locations knees, hips, hands and spine<sup>6,7</sup>. Furthermore, a study based on a random population sample of 337 nuclear families from the Framingham area (Massachusetts, USA) shows that at a mean age of 61.2 already 44.1% showed signs of radiographic OA (ROA) of the knee and out of 30 sites scored the mean number of affected joints was 3.4 (SD 5.3). Amongst offspring of these subjects at a mean age 53.9 at the time of radiographic examination 21.6% was affected by ROA at 1 or both knees, scoring a mean number of affected joints of 1.4 (SD 2.9)<sup>8</sup>. Although the correlation between clinical signs and radiological signs is not strong, both features of OA increase with age<sup>9</sup>. The growing number of elderly in the general population will increase the influence of OA on worldwide public health in the future as illustrated by a prospectus from the Dutch institute for health (RIVM) which predicts the incidence of OA to increase by as much as 52% from 2007 to 2040<sup>10</sup>.

## 1.2 Healthy cartilage

Healthy articular cartilage is characterized by sparsely divided chondrocytes in their extracellular matrix (ECM). The ECM is build up by several classes of highly specialized proteins illustrated in Figure 1. The cartilage matrix is laid down during early development and the major proteins present in matured articular cartilage are collagens, a class of fibrillar proteins. Type II collagen is the major constituent, with a lower abundance of type IX and XI present. Collagens form fibrillar networks that mediate the resistance to compressive forces and provide tensile strength. Furthermore, collagens serve as scaffolding for proteoglycans, the second largest class of proteins present in the ECM.

These polyanionic aggregates are formed by sulphated aggrecan monomers linked by linking proteins to hyaluronic acid. The charged acid proteins help to retain fluid within the matrix, thereby contributing to the ECM integrity<sup>11,12</sup>. The surface of the articular cartilage is smooth and provides a gliding surface allowing low friction movements under high loads.



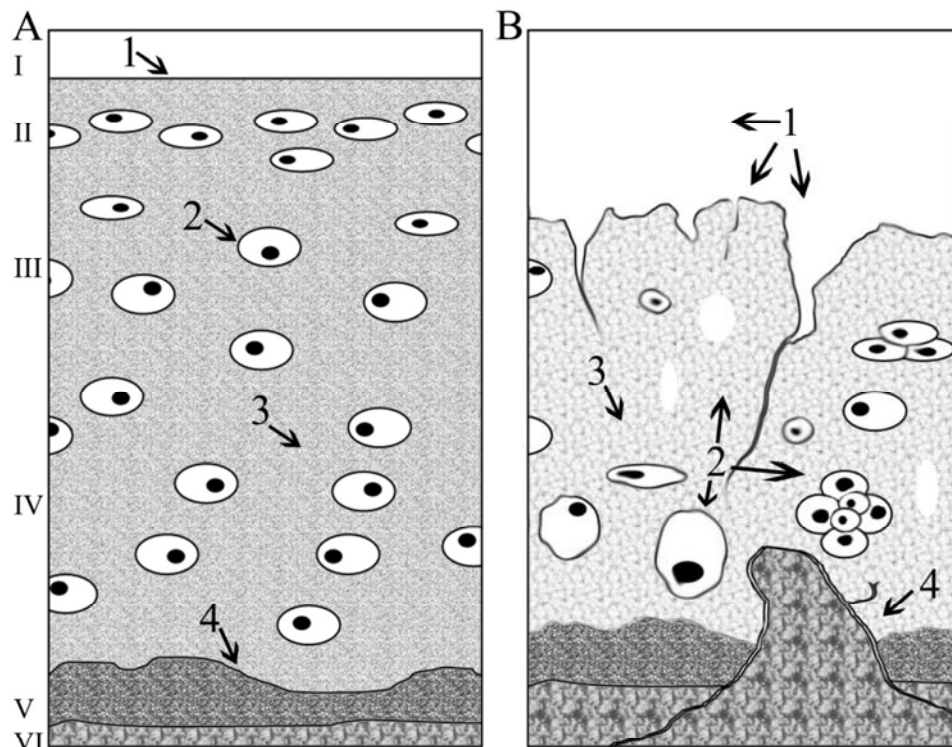
**Figure 1.** Major extracellular matrix constituents with a collagen fibril network (A), proteoglycans chains (B) and side chains with hyaluronic acids (C). These proteins are laid down during development and maintained by sparsely divided chondrocytes (D). The polyanionic side chains help to retain interstitial fluid in the matrix (E).

During development chondrocytes arise from the mesenchymal stem cell layer in the growth plate. Unlike the chondrocytes in the growth plate which develop into bone, the chondrocytes on the joint surface side enter a state of maturational arrest during their development which maintains these in their differentiated phenotype throughout life under physiologic conditions<sup>13</sup>. The difference between the maturational states of the growth plate chondrocytes and articular chondrocytes is mediated through several pathways and biologic

conditions, in which the Wnt-signaling plays a major role<sup>14</sup>. In addition to molecular signaling to maintain their differentiated phenotype, biomechanical activity and hypoxia are essential. The fluid movement, as a result of biomechanical activity, allows nutrients to enter and waste products to exit the matrix in addition to the diffusion processes<sup>15</sup>. The ECM is not perfused by blood or lymphatic systems, ensuring the hypoxic state with oxygen tension as low as 1%, which is needed to maintain the chondrocytes in their differentiated state<sup>16</sup>. Under healthy conditions the chondrocytes have a low metabolic activity while maintaining the ECM<sup>17</sup>. During life, the quality of articular cartilage may lessen as a result of repeated stress and micro fractures to the collagen matrix, accumulation of advanced glycation end products and changes in the cellular expression patterns as a result of aging in general<sup>18,19</sup>. Although the capacity to resist high peak stresses may be less, aged cartilage may still fulfill its role without any compromises to the joint laxity and mobility as long as the glycosaminoglycans stay entrapped in the collagen network and osmotic potential is retained.

### 1.3 OA cartilage

Macroscopic features of OA cartilage include an overall decrease of cartilage volume, fibrillation of the surface, vascularisation, calcification and tears. The accompanying chondrocyte features range from increased metabolic activity, cell cluster formation, dedifferentiation, hypertrophy and ultimately apoptosis<sup>12,17</sup>. Although the order in which the changes occur may differ from case to case, in all instances OA results in an overall cartilage loss and is accompanied by pain and lessening of joint laxity. Frequent observation in early OA chondrocytes are increased metabolic activity and proliferation accompanied by production of matrix degrading enzymes such as matrix metalloproteinases (MMPs) and aggrecanases<sup>1</sup>. The enzymatic activity of these proteins breaks up the tight collagen network of the ECM and shortens the aggrecan side chains, jeopardizing the matrix integrity. The weakened matrix is less capable of retaining fluid and loses its elasticity, decreasing the potential to perform its shock absorbing properties. The matrix becomes more prone to tears and micro fractures, which in turn demands more matrix maintenance from the chondrocytes and allows release of hypoxia at fracture sites by the resulting access to the synovial fluid. Unable to respond adequately to this higher demand the chondrocytes proliferate, turn hypertrophic, dedifferentiate and ultimately become apoptotic<sup>12,13</sup>. Once initiated, this cycle may amplify itself by rounds of matrix weakening and a mainly catabolic or apoptotic response from the chondrocytes. A more macroscopic change often observed in OA cartilage is vascularisation of the matrix from the subchondral bone. This loosens the hypoxic state in the articular cartilage as a result of perfusion allowing phenotypic changes of the chondrocytes, which can initiate formation of bone, illustrated by the calcification of matrix surrounding vascularized areas and formation of osteophytes<sup>20</sup>. Bone is also implicated as an endocrine organ which may locally communicate with the articular chondrocytes, possibly resulting in detrimental processes in response to biomechanical overload or excessive local pressures<sup>21</sup>. Immunohistochemistry can be used to identify different OA features and the accompanied changes observed in matrix and chondrocytes are schematically represented in Figure 2. These features are summarized in a microscopic scoring system described by Mankin *et al.*<sup>22</sup>, which allows quantification of cartilage damage.



**Figure 2.** Schematically represented the synovial fluid (I), cartilage of the upper (II), middle (III) and deep (IV) region, a calcified area (V) and cancellous bone (VI). (A) In healthy cartilage a smooth running surface lines the boundary of the tissue with the synovial fluid (1). Cells are dispersed evenly over the tissue and are in a quiescent state (2). The proteins of the extracellular matrix, consisting mainly of collagens and glycosaminoglycans retain fluid and provide tensile strength (3). A tidemark separates the cartilage from the calcified area and cancellous bone. (B) In OA, the overall thickness of the cartilage is lost, the surface becomes irregular with fractures (1). Cellular features of OA include hypocellularity, clonal expansion of the chondrocytes and chondrocyte hypertrophy (2). The proteins of the extracellular matrix become degraded, losing their elasticity, tensile strength and fluid retaining properties (3). The tidemark becomes less clear and is crossed by blood vessels (4).

#### 1.4 Heritability and genetics of OA

Family studies have shown that a relatively large heritable component exists in OA, with heritability estimates ranging from 40-80%, depending on phenotype and joint site studied<sup>23-25</sup>. Already in the 19<sup>th</sup> century OA features were recognized as hereditary and a 1941 study reported OA as a dominant trait<sup>26</sup>. Over the years OA has shown to have a complex genetic background. In comparison to other common diseases OA reports a high familial clustering with recurrent risk ratio's, depending on subtype studied, between 1.66 and 8.53<sup>24,27,28</sup>. Given these data it is apparent that genes play a substantial role in OA and although some rare mutations cause a monogenic early onset form of OA<sup>29</sup>, the majority of the heritability component is caused by a multitude of genetic factors<sup>30,31</sup>. The complexity of the disease makes the identification of genes involved a meticulous exercise where large well typed cohorts with high genetic resolution are needed to yield enough power to elucidate the underlying genetic background<sup>32</sup>. To date several genome wide approaches, as well as candidate gene studies have delivered multiple genes which show reasonably



consistent contributions to the disease etiology. A list of recent and striking studies in the genetics of OA, which were at least confirmed once or show robust numbers is supplied in Table 1. Identification of genes involved in a trait can be done through several approaches, each of which has advantages and disadvantages over the others and optimal study designs depend entirely on the frequency and penetrance of the genetic variation underlying the disease (Figure 3).

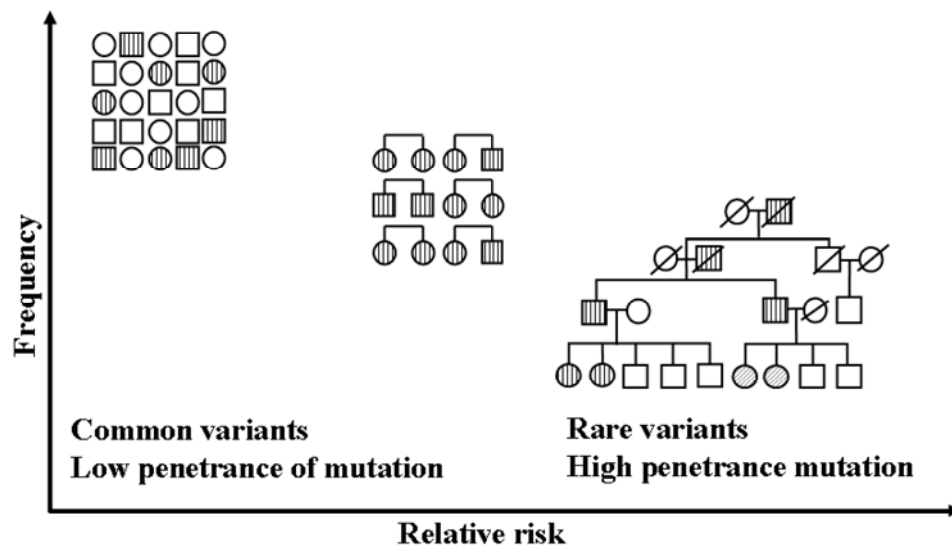


Figure 3 Genetic study designs

Rare mutations with large effect sizes are best identified by model based linkage in extended families where a Mendelian inheritance pattern indicates a single gene to be the major factor in disease onset. Model free linkage studies in affected sibling pairs may be the next approach to detect variants with moderate effects on disease etiology for which the inheritance pattern appears more complex. An advantage over the association studies is the resistance to genetic heterogeneity and the power to identify genetic loci which may carry multiple independent variants which influence disease etiology. Association studies rely on the sharing of ancestral disease associated alleles with lower penetrance and higher allele frequencies amongst cases versus controls, referred to as the common disease, common variant theory<sup>33</sup>. Initially, candidate gene approaches were performed, where based on knowledge of the disease process, the choice to characterize a specific gene used little genotyping resources. Obviously, this only informs the researchers on a specific locus thereby not identifying other genetic loci putatively involved in the disease. Technological advances enabled researchers to type increasingly more variants, up to a point where a genome wide and thereby unbiased approach could be taken and a discovery approach was possible next to the hypothesis based approaches. In genome wide approaches we distinguish a linkage approach, which is based on family studies and association approaches which are population based, identifying cases and controls and comparing their allele frequencies. The genome wide approaches suffer from large numbers of spurious signals, increasing with the number of tests performed. To account for this, a correction for multiple

testing is applied, which requires the signals to reach a 'genome wide significance' level of  $P < 1 \cdot 10^{-8}$  before being considered as putative true signals. Although combining several studies in collaborative efforts to accumulate larger numbers in the genome wide studies is generally beneficial, there is an increased chance of heterogeneity of the study population. Especially in the case of a heterogeneous disease such as osteoarthritis such heterogeneity may prevent identification of specific risk genes, cancelling out the beneficial effects of the large combined study sample. Further challenges in the unbiased approaches are the lack of incorporation of putative gene-gene and gene-environment interactions<sup>33</sup>. Once a particular gene is identified as involved in a disease etiology, much work is needed to understand the underlying mechanism. A thorough characterization of the genetic region is needed to identify the (true) causal variant and quantify its contribution to the disease susceptibility and gain understanding of the involved molecular pathways. The recently developed second generation sequencing techniques now allow researchers to sequence a substantial genetic area in large numbers of subjects at a reasonable cost, facilitating the identification of new variants in these areas of interest<sup>34</sup>, which is illustrated by the recent study by Johansen *et al*, who showed an accumulation of rare variants at a series of candidate genes in hypotriglyceridemia cases versus controls<sup>35</sup>.

Altogether, amongst the results of genetic studies performed in OA (subtypes) two distinct pathways stand out especially with multiple genes implicated. The first group is formed by the inflammatory pathway with inflammatory signaling proteins, their modulators, receptors and the inflammation driven induced degrading enzymes such as matrix metalloproteinases (MMPs) and aggrecanases. Some consistently associating genes from this group are the interleukin (IL)-1 gene cluster<sup>67-69</sup>, interleukins<sup>72,77</sup>, aggrecanases<sup>37,80</sup> and prostaglandin<sup>36,37,62</sup>. The second group identified consists of genes involved in the developmental processes of chondrogenesis and osteogenesis such as Wnt-signaling proteins and bone morphogenic proteins. Some of the compelling genes in this group are *FRZB*<sup>41,49,94</sup>, which codes for an antagonist of the Wnt-signaling, *GDF5*<sup>55</sup> which coordinates formation of bone and joints, *BMP2*<sup>37</sup> and *BMP5*<sup>44,45</sup>, regulators of chondrogenesis and articular cartilage formation respectively. Although not all genes are replicated in every study, the overall view is that these developmental genes play a role in the disease etiology.

### **1.5 The role of inflammatory mediators in OA**

Articular chondrocytes respond to specific cytokines by either anabolic or catabolic activity. Pro-inflammatory signaling mediated by e.g. IL-1 $\beta$  has detrimental effects on cartilage since the resident chondrocytes respond by producing MMP's and aggrecanases, effectively degrading the surrounding matrix. On the other hand, signaling by anti-inflammatory cytokines such as IL-10 results in anabolic activity of the chondrocytes which will excrete new ECM components. Under normal, healthy conditions the pro- and anti-inflammatory signals are low and balanced thereby cancelling out their respective effects<sup>95-97</sup>. This balanced mechanism is thought to play a role in repair of small traumas to the cartilage, where catabolic activity in response to pro-inflammatory signaling, resulting from the sensed trauma, clears the damaged matrix<sup>98,99</sup>. Subsequently, the resulting lacuna in the matrix is filled by the newly formed collagen matrix in response to the later stage induced anti-inflammatory signaling<sup>12,95,100</sup>. The cytokine response is, at least in part, under genetic control as is illustrated by several studies showing associations of circulating levels of

**Table 1.** Recent and confirmed genetic studies for OA susceptibility loci.

Pathway	Gene	Studies +	Studies -	OA subtypes	Gene description and function
Developmental	<i>AACT</i>	36,37		Knee	Alpha 1 antiproteinase, extracellular antagonist of the Wnt-signaling pathway
	<i>ASPN</i>	38-41	42,43	Hip, Knee	Asporin, Cartilage extracellular protein that regulates TGF- $\beta$ signaling
	<i>BMP2</i>	36,37		Knee	Bone morphogenetic protein 2, involved in the chondrogenesis and osteogenesis in development
	<i>BMP5</i>	44,45		Knee	Bone morphogenetic protein 5, regulator of articular chondrocyte development
	<i>CILP</i>	36,37,46		Hip, LDD	Cartilage intermediate layer protein, inhibits TGF- $\beta$ 1-mediated induction of cartilage matrix genes
	<i>DIO2</i>	47		Hip, GOA	Deiodinase 2, intracellular activator of thyroid hormone in target tissues
	<i>DIO3</i>	Meulenbelt <i>et al.</i> (in press)			Deiodinase 3, intercellular deactivator of thyroid hormone in target tissues
	<i>FRZB</i>	41,48-51	52	Hip/Knee, GOA	Secreted frizzled related protein 3, Wnt antagonist and modulator of chondrocyte maturation
	<i>GDF5</i>	53-55		Hip	Growth and differentiation factor 5, member of the bone morphogenic family, regulator of growth and differentiation across several tissues including bone and cartilage
	<i>IGF1</i>	43,56		Hip	The protein is similar to insulin in function and structure and is a member of family of proteins involved in mediating growth and development.
	<i>LRP5</i>	57	52	Knee	Low density lipoprotein receptor-related protein 5, receptor involved in the Wnt signaling via the canonical beta-catenin pathway
	<i>RHOB</i>	58	59	Hip, Knee	Ras homologue gene family member B, GTPase in tumor suppression and antagonist in the PI3K/Akt pathway
<i>WISP1</i>	60		Spine	Wnt inducible signaling protein 1, target of the Wnt pathway regulated by beta catenin	
Inflammatory and Immunity	<i>COX2 (PTGS2)</i>	36,37,61,62		Knee, Spine	Prostaglandin, modulates cartilage proteoglycan degradation through PGE2
	<i>HLA</i>	63-66		Hand/Hip/Knee, GOA	Human leukocyte antigen system, determinant of antigen specificity of the immune response
	<i>IL1 cluster</i>	43,67-70	43,69,71	Hip/Knee	Interleukin 1 alpha, beta and receptor antagonist, regulates metalloproteinase gene expression in chondrocytes and synovium
	<i>IL10</i>	72,73		Knee/Hand	Interleukin 10, anti inflammatory cytokine inhibiting IL1 synthesis
	<i>IL4R</i>	74	43	Hip	Interleukin 4 receptor, Putative role in chondrocyte response to mechanical signals
	<i>IL6</i>	75-77	43	Hip/Knee	Interleukin 6, proinflammatory cytokine, involved in cartilage degradation and IL1Ra induction
Structural/Other & Undefined	<i>Chr7q22</i>	78		OA subtypes	Several genes in LD block currently under investigation for further specification of underlying gene(s).
	<i>ADAM12</i>	31,37,79	42	Knee	A Disintegrin and metalloproteinase 12, involved in osteoclast formation and cell-cell fusion
	<i>ADAMTS14</i>	80			A disintegrin and metalloproteinase with thrombospondin motifs 14
	<i>CALM1</i>	81	41,82	Hip	Calmodulin 1, intracellular protein interacting with proteins involved in signal transduction
	<i>COL2A1</i>	41,83-85	43	Knee	Type II collagen, major constituent of articular cartilage
	<i>COMP</i>	41		Knee	Cartilage oligomeric protein, cartilage matrix macromolecule
	<i>EDG2</i>	86		Knee	Lysophosphatidic receptor encoding, EDG receptors mediate diverse biologic functions, including proliferation and chemotaxis.
	<i>ESR1</i>	36,87,88	43	Knee, GOA	Estrogen receptor alpha, chondrocytic modulator of proteoglycan degradation and MMP mRNA expression
	<i>LRCH1</i>	89	90	Hip/Knee	Leucine rich repeats and calpain homology, protein of unknown function
	<i>MATN3</i>	91,92		Hand, Spine	Matrillin 3, extracellular matrix protein
	<i>OPG</i>	36,37		Knee	Osteoprotegerin, regulator of osteogenesis
	<i>TNA</i>	36,37		Knee	Tetranectin, plasminogen-binding protein involved in the degradation of ECM
	<i>TXNDC3</i>	58	59	Knee	Thioredoxin domain containing 3, protein disulfide reductase
<i>VDR1</i>	36,41,59	43,93	Knee	Vitamin D receptor, mediates vitamin D signaling which serum levels affect incidence and progression of OA	

inflammatory mediators to genetic variation at genes involved in their regulation<sup>101-103</sup>. Cytokines are derived from several cell types, however, one of the main sources of cytokines are the circulating blood lymphocytes. The estimated innate capacity of lymphocytes to produce cytokines has been shown to associate to a range of diseases<sup>104,105</sup> including OA susceptibility and OA progression in sibling pairs affected by OA at multiple joint sites (Genetics of osteoARthritis Progression, or GARP study<sup>106</sup>). In this study, Riyazi *et al.* showed that a high innate IL-1 $\beta$  and low innate IL-10 (together indicating a pro-inflammatory profile) predisposes to OA<sup>107</sup>. Furthermore, data on progression of a subset of these individuals over a 2 year period showed a specific innate tumor necrosis factor(TNF) $\alpha$  and IL-10 profile confers to an increased risk of knee OA progression<sup>108</sup>. The genetic component in variation of the LPS induced innate cytokine response is substantial, underlined by the high heritability of these levels ranging from 57% to 86%<sup>109</sup>. Genetic configuration of innate immunity might confer susceptibility to OA through a lifelong exposure to specific (pro-inflammatory) cytokine profiles<sup>107</sup>, as is also observed for several other diseases<sup>110,111</sup>. Polymorphisms in the cytokine genes were shown to explain only a small part of the total heritability estimate of innate immune profiles indicating involvement of additional loci<sup>73,109</sup>. The role of polymorphisms at the *IL-1* gene cluster in the innate levels or OA phenotypes were studied in several smaller cohorts and to date no study combined the polymorphisms, innate levels and OA data in one single study. In addition, the ratio between IL-1 $\beta$  and its biological competitor IL-1 receptor antagonist (Ra) was suggested to be a more suitable representation of the bio-availability of IL-1 $\beta$  signal<sup>112</sup>. The low contribution of the known genetic variation at the respective cytokine genes to the genetic component of innate immunity indicates additional regulating mechanisms are involved. Identification of new loci involved in the regulation of innate immunity may provide new candidate genes to be tested for association to OA. In addition to innate immunity, synovial levels of inflammatory cytokines were reported to be elevated in OA joints<sup>97,113-115</sup>, whereas baseline levels of C-reactive protein (CRP) were shown to be associated to erosive hand OA<sup>116</sup>. Furthermore, *in vitro* explants and cell culture experiments show that the chondrocytes are a source of matrix degrading enzymes upon incubation with pro-inflammatory cytokines<sup>117-120</sup>. Taken together the described studies show that inflammatory mediators play a role in OA etiology, although for most reported studies it remains unclear whether the immune system signaling is merely a marker of the ongoing disease process, or whether this may underlie part of the etiology of the disease. Studies into genetics of the immune system may help elucidating cause and effect. As was shown in Table 1, in addition to the genetic component in OA that may be ascribed to genetic variation in inflammatory mediators, recent genome wide linkage and association scans also indicated a substantial role for genes which play a role during early development of the articular joints.

### **1.6 The role of developmental characteristics in OA**

The role of skeletal development in OA was first illustrated by the onset of OA as a result of severe chondrodysplasia or defects in skeletal morphogenesis<sup>121,122</sup>. The joint shape is determined early in life, where severe malformations ultimately lead to OA. The endochondral ossification process plays a key role in determining joint shape during development and is responsible for the growth and shape of the long bones, including the articular joints<sup>14</sup>. In this process chondrocytes arising from a mesenchymal stem cell layer

subsequently proliferate, differentiate and turn hypertrophic. During these phases respectively, the chondrocytes increase in number, lay down a cartilage matrix and eventually start excreting cartilage degrading enzymes, and ultimately the initial cartilage layer is replaced by bone by blood vessel invasion and settlement of osteoblasts. The chondrocyte phases are tightly controlled by distinct gene expression patterns in which the Wnt-signaling is intimately involved<sup>123</sup>. Contrary to the chondrocytes in the growth plate, the chondrocytes of the articular layers on the long bones escape this sequence and enter a state of maturational arrest maintaining their differentiated phenotype. It can be hypothesized that less pronounced joint shape variations can predispose to OA as a result of a lifelong slightly altered mechanical loading of the joint. Several studies are ongoing in an attempt to define joint shape by the use of radiographs or MRI and relating this to the incidence of OA. Although confirmation is needed, initial studies show joint shape modeling may be used to predict OA onset or progression<sup>124,125</sup>. Through a genome wide linkage scan in the GARP study, a new OA susceptibility locus was identified at 14q32.11<sup>47</sup>. Subsequent association analysis identified the local thyroid hormone regulator *DIO2* coding for type II deiodinase (D2), which is also active in the growth plate during development as the most likely gene in the linkage region involved in OA etiology. Genetic variation at *DIO2* was confirmed to associate to OA in multiple other studies across different geographic areas and ethnicities. *DIO2* is the first gene identified which indicated that thyroid signaling may be involved in OA and secondly that such signaling in the growth plate during endochondral ossification may be involved in OA etiology<sup>126</sup>. Several other genetic studies likewise identified genes in the osteogenesis and chondrogenesis pathways that associate to OA<sup>41,55</sup>, indicating a broader involvement of genes which orchestrate the process of endochondral ossification. In addition to developmental differences originating from developmental stages these genes may contribute to OA etiology later in life; the processes observed in the articular chondrocytes and ECM resemble processes observed in the growth plate in osteogenesis<sup>95,127</sup>, possibly the (aging) articular chondrocytes lose their maturational arrested phenotype. Loss of this arrest allows articular chondrocytes to re-enter the cycle of their counterparts in the growth plate, thereby mimicking the downstream events and ultimately turning apoptotic and causing the cartilage integrity to fail. We have discussed the features of ageing and the role of developmental genes in OA in a review on this subject<sup>126</sup>.

### **1.7 Outline of the thesis**

In the current thesis one of the main study populations that is being investigated is the GARP study, consisting of middle aged sibling pairs (total N=382) affected with OA at multiple joint sites. This study is characterized for OA features at four joint sites; OA at the hips, knees and hand and degeneration of spinal discs were assessed by radiographs as well as by clinical assessment<sup>106</sup>. A high level of familial clustering of these features was observed in the GARP study, which led us to identify the genes underlying this high heritable component in OA. In addition to radiological and clinical data for these joint sites, extensive demographic questionnaires, blood samples and urine were collected at different time points (0, 6, 12, 24 and 60 months). The collection of multiple time points allows studies aimed at identifying markers which can either show or predict OA progression. Genetic studies in the GARP study by Min et al. have thus far shown associations to *FRZB* and *MATN3* for hand and hip OA respectively<sup>51,91</sup>. The associations of the described genes

in the GARP study together with the results of genetic studies in other OA cohorts explain only part of the heritability of OA, indicating that additional genes are involved in OA etiology.

In this thesis we set off to gain more insight in the role of inflammatory mediators and their genetic variation in OA. The question to be answered is whether (innate) cytokine profiles are merely a reflection of ongoing OA processes and genetic configuration, or whether the genetic configuration of genes orchestrating these cytokines plays a role in OA etiology. We characterized several genes and inflammatory markers and investigated their respective roles in OA in order to elucidate whether the observed associations follow Mendelian randomization<sup>128</sup>, or whether an etiologic relation exists between genetics of cytokine regulating genes and OA. Chapter 2 describes the research performed in the GARP study to substantiate the causal role of inflammatory mediators and inflammation modulating genes in OA.

In Chapter 2.1 we studied the interaction between genetic variation at the IL-1 gene cluster (*IL1A*, *IL1B* and *IL1RN* gene) and innate *ex vivo* production upon LPS stimulation of the IL-1 $\beta$  and IL-1Ra (together expressing the IL-1 $\beta$  bio-availability) as well as the interaction of these characteristics with OA. Although genetic variations associated to innate cytokine levels were found as described Chapter in 2.1 of this thesis and in literature, together these genes explain only a small part of the heritability of these levels and elucidation of quantitative trait loci for innate immunity might reveal new insights in the regulation of the cytokine response. Follow up on these putative innate modulating genes may provide more insights in the role of innate immunity in OA etiology. Chapter 2.2 describes a genome wide linkage study where we set off to identify new quantitative trait loci for innate IL-1 $\beta$ , IL-1Ra, IL-10 and TNF $\alpha$  and whether these associate to OA. In Chapter 2.3 we explored the association between *CRP* haplotypes and serum high sensitive CRP levels as shown by Carlson *et al.*<sup>102</sup> and used these haplotypes and CRP levels in an association analysis with OA subtypes. Finally in Chapter 2.4 we investigated whether serum CRP and plasma cytokine and/or chemokines levels marked the ongoing OA disease process in the GARP study. Furthermore, using these levels we investigated whether the previously identified association between genetic variation at the *SELS* gene and pro-inflammatory cytokines<sup>101</sup> in plasma could also be identified in the inflammatory profiles of GARP subjects and their OA subtypes.

As indicated, previous studies identified the *DIO2* gene as the most likely gene explaining a significant linkage peak in OA sibling pairs of the GARP study<sup>47</sup>. A hypothesis on the role of genes involved in endochondral ossification was generated during the research of this thesis<sup>126</sup>. We characterized a *DIO2* risk allele tagged by polymorphism rs225014 for possible *cis* regulatory elements through analyses of putative differential allelic expression and we sought further evidence for the involvement of D2 in OA by characterization of OA and non-OA cartilage for the presence of D2 including other thyroid hormone regulatory proteins by use of immunohistochemistry described in Chapter 3.

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