Generalised Osteoarthritis: from Mendelian Disorder to Complex Disease

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CHAPTER 1

General introduction

1.1 Classification and epidemiology of osteoarthritis

The term osteoarthritis (OA) describes a common, age-related, heterogeneous group of musculoskeletal disorders. The pathology of osteoarthritis involves the whole joint and is characterised by focal areas of loss of articular cartilage in synovial joints, associated with varying degrees of osteophyte formation, subchondral bone changes, and synovitis. Joint damage is caused by a mixture of systemic factors that predispose to the disease, and local mechanical factors that dictate its distribution and severity[1;2]. Classically, the diagnosis of OA in epidemiological studies has relied on radiographic characteristics described in 1957 by Kellgren and Lawrence[3] and illustrated in the Atlas of Standard Radiographs[4]. As illustrated in Figure 1 and 2, the main radiographic characteristics include joint space narrowing (JSN), subchondral sclerosis and osteophytes reflecting the underlying processes of cartilage loss, bone change, and new bone formation respectively. On the basis of combination of these changes, the widely used ordinal grading scheme (0-4) for severity of radiographic features of OA (ROA) was developed[4].

OA can occur in any synovial joint in the body but arises most frequently in the lumbar and cervical spine, specific hand joints (distal interphalangeal joints (DIPs, proximal interphalangeal joints (PIPs) and first carpometacarpal joints (CMC1)), knees and hips. A single joint can be affected (localised OA) or several or multiple joints are affected (generalised OA). Epidemiological population surveys in the elderly have supplied prevalence, incidence and risk factors of ROA[5-10].

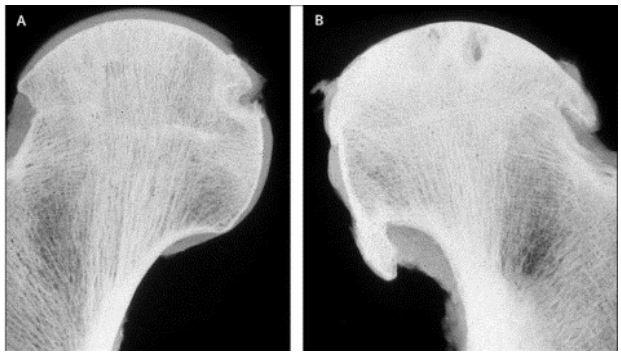

Figure 1 Radiograph of (A) normal and (B) osteoarthritic femoral head.
Radiograph of osteoarthritic joint shows marginal osteophytes, change in shape of bone, subchondral bone cysts, and focal area of extensive loss of articular cartilage. (Source: Dieppe et al., Lancet 2005, with permission.)

One of those surveys, used in this thesis, is the prospective population-based Rotterdam study comprising 7,983 Caucasian inhabitants of a suburb of Rotterdam aged 55 years and older[10]. In a random sample, comprising 55-65 years old participants of the Rotterdam study, 81% of the men and 85% of the women, showed ROA in any of the four joint sites (hand, hip, knee, spinal disc degeneration (DD)) scored. High joint specific prevalences of ROA occurred in spinal discs (61%), hand (56%), knee (18%), and hip (9%). ROA in more than one joint site (47%) was also very common[11].

Several US and European studies indicated that the incidence of hand, hip and knee ROA is high and strongly rising with age, especially after the age of 50 and is higher in women than in men[12-15]. In addition to increasing age and female gender, other systemic risk factors include genetic factors, bone density and obesity[15]. Local risk factors of OA include joint injury, muscle weakness, malalignment and developmental deformity. The impact of such risk factors in different joints vary[15;16]. Clinically, OA is characterised by joint pain, tenderness, limitation of movement, crepitus, occasional effusion and variable degrees of inflammation without systemic effects[17]. However, clinical symptoms and signs often correlate poorly with the radiographic changes. For purposes of clinical research, the American College of Rheumatology (ACR) has developed reliable classification criteria for hip[18], knee[19] and hand[20] OA with a high level of sensitivity and specificity. These criteria rely on combinations of symptoms, physical findings, laboratory data, and radiographic

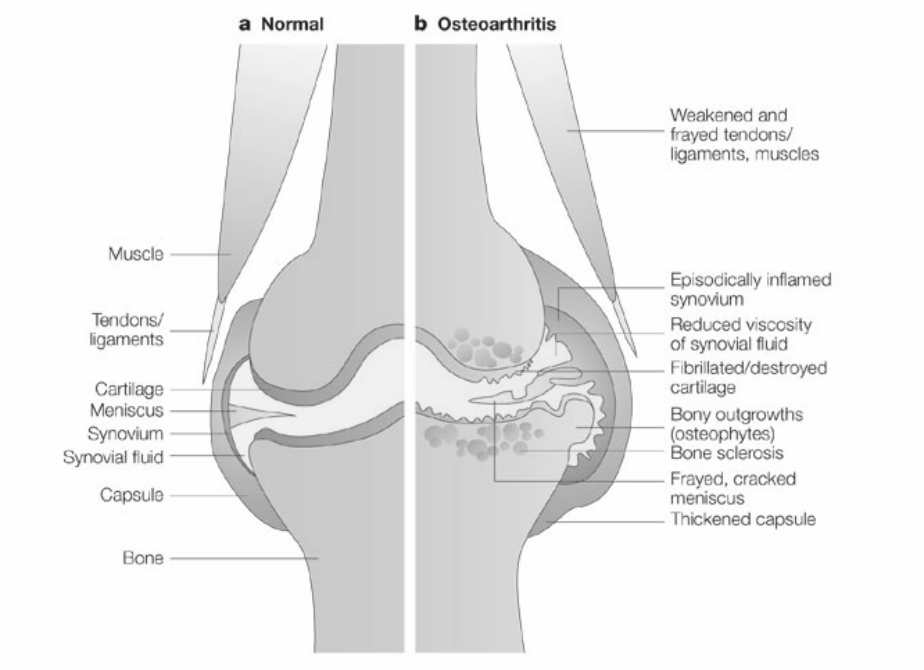

Figure 2 Schematic overview of a normal joint (A) and an osteoarthritic joint (B).
(Source: Wieland et al., Nat Rev Drug Discov 2005, with permission.)

features[17]. The prevalence of symptomatic OA is considerably lower than ROA because of the high proportion of subjects with ROA who do not have joint pain. Prevalences in adults aged 25-74 years varied between 1.9% for knee OA and 2.4% for hand OA. In elderly, prevalences have ranged between 0.7-4.4% for hip OA and 10-15% for knee OA[7;12;15;21].

OA is now recognised as a group of overlapping distinct diseases, which may have a wide variety of different pathological processes but with similar biologic, morphologic, and clinical outcomes[22]. The diagnosis of OA based on a combination of symptoms and radiology (such as used in ACR criteria) is a difficult phenotype for biological studies since it likely reflects a range of pathological processes. Initially, classification of OA into primary and secondary OA was based on the absence or presence of causative factors respectively. However, improved understanding of the disease suggests that these classifications can not be maintained. OA is a common complex disease with both environmental and genetic determinants. Environmental factors such as joint injury may be sufficient to cause OA. Conversely, genetic factors predominate in families transmitting OA as a rare Mendelian trait with early onset. Most individuals with OA are probably affected by the interplay between genetic and environmental factors[1;16].

Of specific interest for this thesis, is the co-occurrence of OA at multiple joint sites. This form of OA may especially reflect the effect of systemic factors such as genetic factors. As early as in 1952, Kellgren and Moore classified generalised radiographic OA (GOA) in middle-aged women for those with three or more affected joints or joint groups (knee, hip, hand)[23]. They subclassified GOA, on the basis of either the presence or absence of Heberden's nodes (nodal or non-nodal GOA) and considered GOA as a distinct clinical entity[23]. Epidemiological studies provided further evidence for the existence of radiographic GOA. Multiple joint involvement occurred more frequently than would be expected from the rising prevalence of OA with advancing age or female sex[24-27]. To date, various GOA definitions exist and rely for example on: joint site (e.g. the smaller hand joints only, hip and knee joints only, four joint sites including spinal discs, typical and atypical sites including ankle, shoulders or elbows), clustering of affected joints or a threshold or sumscore of the number of affected joint sites[28;29]. In this thesis, the definition of GOA is based on two or more joint sites affected (hand, spinal DD, hip or knee).

In several studies, clustering patterns of joint involvement have been investigated to improve the definition of GOA[24-27]. The most striking patterns of clustering were observed for multiple joint involvement of the hand. Clustering occurred often symmetrically (bilateral involvement), and is more likely to be clustered by joint (e.g. DIPs) than by ray (e.g. finger)[25]. Together, the definition of GOA still has no consensus among clinicians and researchers. Consequently, problems arise during interpretation (see below) and comparisons between studies.

The principal aim of this thesis is to gain insights into the aetiology of OA at multiple joint sites by the identification of gene variants that contribute to the susceptibility of familial rare and common GOA and common OA in the population. The identification of specific and common genetic variants that confer increased risk for OA susceptibility will improve our understanding of the disease processes involved in OA. In addition, these variants may provide targets for development of new forms of treatment.

1.2 Pathogenesis of osteoarthritis

Articular cartilage is a highly specialised and uniquely designed bio-material that forms the smooth, gliding surface of the diarthrodial joints. It is an avascular, aneural and alymphatic matrix which is synthesised by the sparsely distributed resident cells, the chondrocytes[30]. The extracellular cartilage matrix (ECM) consists of a fibrillar and extrafibrillar component. The fibrillar matrix predominately consists of type II collagen but comprises also other collagens mainly type IX and XI. The nonfibrillar component contains mainly highly sulphated aggrecan monomers attached to hyaluronic acid via link proteins, forming very large polyanionic aggregates (Figure 3). In cartilage, the collagen network confers tensile strength whereas the aggrecan component provides compressive stiffness to articular surfaces. Under compressive force, cartilage matrix is compliant, when water is

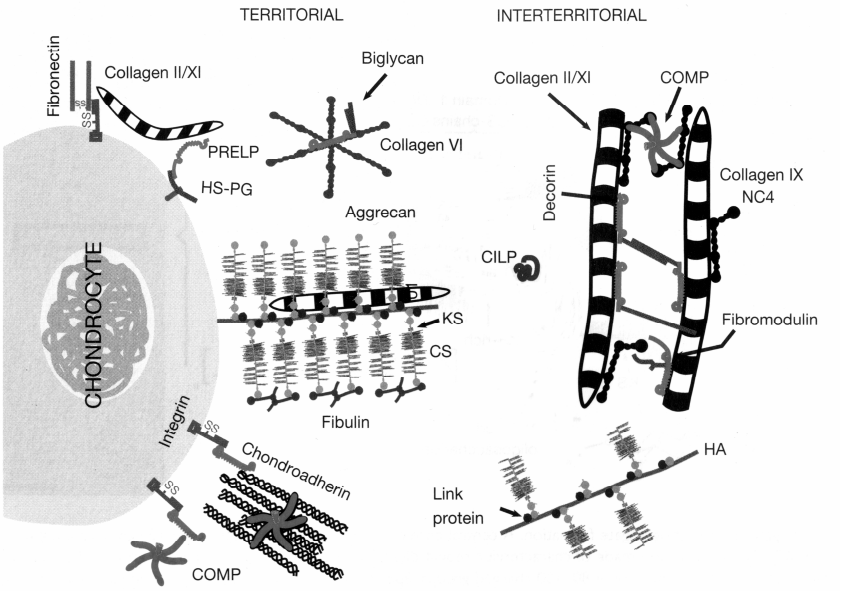

Figure 3 Schematic illustration of the constituents of the extracellular cartilage matrix (ECM). The assembly and aggregates with extracellular matrix components are depicted. The ECM of cartilage consists of a territorial or pericellular region and an interterritorial region further away from the cell. (Source: Heinegard et al. in Osteoarthritis 2nd ed., with permission.)

drawn back into the matrix by the hydrophilic aggrecan aggregates. As illustrated in figure 3, several other matrix components play important roles in maintaining cartilage function and structure for example fibronectin, matrilin-3 and cartilage oligomeric matrix protein which might be involved in matrix cohesion and chondrocyte regulation[2;31].

OA appears to be a result of an imbalance between synthetic (anabolic) and resorptive (catabolic) activities of the resident chondrocytes in response to mechanical stress. Finally, it results in a net loss of cartilage matrix components and deterioration of the structural and functional properties of the cartilage[32]. At the molecular level, loosening of the collagen network and loss of proteoglycans occur during early OA stages. As yet, it is not known which of these events happens first. The first histological change in OA is cartilage edema in the ECM resulting from the depletion of proteoglycans. As a result, the cartilage becomes softer and, therefore, more susceptible to mechanical injury leading to increased cell death of chondrocytes. In early OA, the chondrocyte exhibits a transient proliferative response, increased synthesis of the ECM as an early attempt to repair, and enhanced synthesis of catabolic cytokines and matrix-degrading enzymes (Figure 4)[2;31;32]. The failure of an adequate response of repair to injury results in irreversible changes in tissue composition leading to hypertrophy and distortion of the joint[2].

An intriguing issue that remains unexplained is whether the pathogenesis of OA at each joint site is identical. All joint tissues are presumably exposed to common systemic stimuli and more joints become affected when OA progresses[2;21;33]. It is, therefore, plausible that some of those factors cause OA at multiple joint sites. Mutations in genes may for example act by making cartilage more susceptible to injuries and less capable of repair[21].

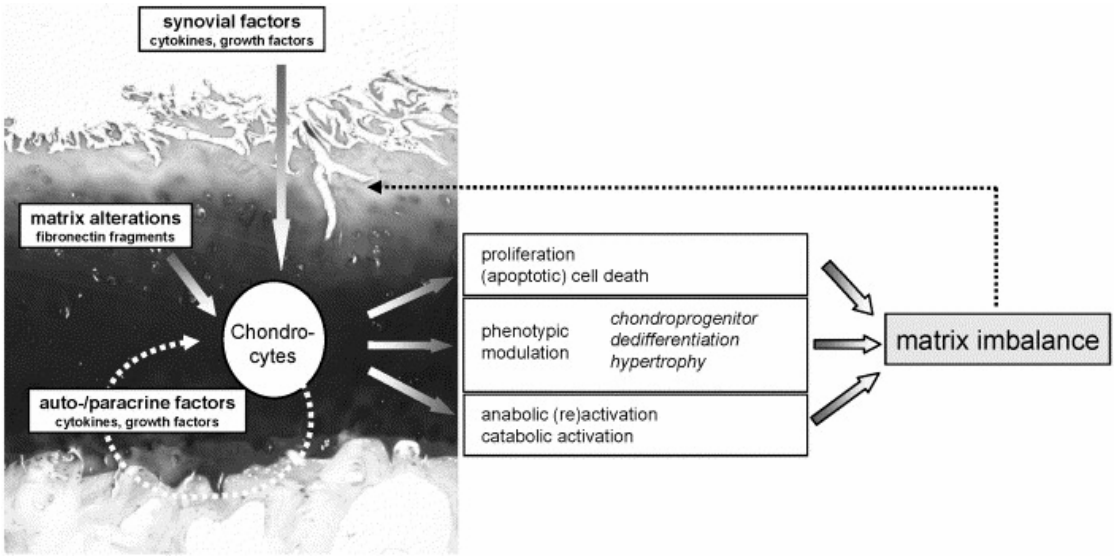

Figure 4 Schematic representation of the response of the chondrocytes.
(Source: Aigner et al. in Adv Drug Deliv Rev 2006, with permission.)

Alternatively, severe joint injury may be sufficient to cause osteoarthritis in a specific joint, some joints are spared from developing OA and prevalences between joint sites differ concisely. The ankle, for example, is much less likely to be affected by OA than the knee. Local risk factors may alter the biomechanics and loading stress conditions of various joints in different ways and may lead to distinct OA entities. Therefore, the question arises whether the pathology of hip OA for example differs from that of a GOA phenotype including hip OA[2;21;33]. It remains unclear to what extent the biological mechanisms and genetic factors are common to the pathogenesis of different OA entities.

1.3 Heritability of osteoarthritis

Clinical observations have long supported the concept that there is some genetic or familial contribution to OA. In 1941, Stecher already noted that Heberden's nodes occurred three times more frequently in the sisters of affected women than in the general population[34]. Kellgren et al. showed that first degree relatives of probands with GOA are twice as likely as population controls to have GOA[23;35]. Familial aggregation is also confirmed for OA at specific joint sites[28;29;36-42]. Familial clustering, however, might be explained by shared genetic but also by shared environmental factors, such as dietary intake, occupation and physical activity. Classical twin studies, in which the relative contribution of environmental and genetic determinants can be differentiated by comparison of the occurrence of ROA in monozygotic twins and dizygotic twins confirmed a substantial genetic contribution

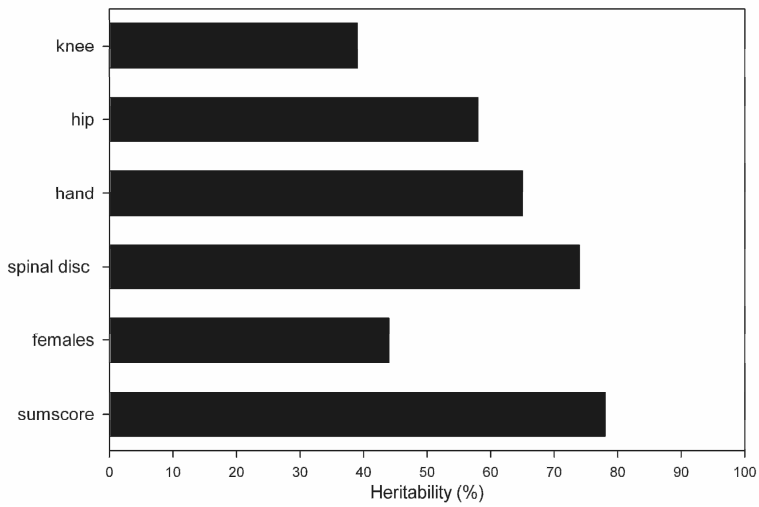

Figure 5 Heritabilities for specific joint sites. Heritabilities of the knee, hip, hand and spine are estimated in twin studies and the sumscore represents the number of affected joints of 36 joint groups (hand, knee, hip and spine) scored.

for the familial clustering of common OA[43-46]. As illustrated in figure 5, twin studies revealed heritabilities ranging between 39% for the knee to 74% of the lumbar spine[43-45]. The heritability appears to be greater in women than in men[46]. Segregation analysis of hand and knee OA (termed GOA) suggested a significant genetic contribution with evidence for a major recessive gene in combination of polygenic effects[28].High familial aggregation estimates for GOA has been observed in the studies used in this thesis. Siblings of probands with radiographic GOA from the population-based Rotterdam study showed high heritabilities of hand ROA (0.56) and disc degeneration (0.75) but no significant evidence for knee or hip ROA[29]. Furthermore, a high heritability (0.78) of a sumscore of the number of affected joint sites was observed (Figure 5)[29].

In the Genetics OsteoARthritis and Progression (GARP) study, which consists of Caucasian probands and their siblings of Dutch origin affected predominantly with symptomatic OA at multiple sites, familial aggregation of osteoarthritis differs by joint site[47]. Siblings of selected probands with clinical GOA tended to be affected at the same joint sites, especially for hip, hand, or spine but not for knee[47]. Overall, genetic factors comprise an essential component of the aetiology of OA which clearly justifies a search for causal genes. The high heritabilities and familial aggregation estimates for GOA phenotypes in addition to the primarily symmetric pattern of involvement of multiple joints strongly suggest a systemic genetic aetiology of OA in at least a part of the patients. These heritability studies indicated significant genetic components in common OA and have prompted the collection of affected sibling pairs with common OA with late-onset.

1.4 Rare families with early onset osteoarthritis

To date, mutations in approximately 1900-2000 genes have been identified to cause disease (OMIM; http://www.ncbi.nlm.nih.gov/Omim/mimstats.html, The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/). Most of these mutations have been described for 'simple' Mendelian disorders in which mutations at one locus are necessary and sufficient for the expressed phenotype. During the last decade, several studies in families have been performed in which OA was transmitted as a dominant trait often with incomplete penetrance. In most of these studies, subjects are affected with osteochondrodysplasias or other rare skeletal diseases that can predispose to OA. Many chondrodysplasias have been attributed to mutations in genes encoding cartilage-specific collagens (*COL2A1*, *COL11A2*, *COL9A1*, and *COL9A2*) and noncollagenous components of the ECM (*COMP*, *FGFR3*, *DTDST*, *MATN3*)[33;48;49].

The successful identification of these mutations has contributed to elucidation of gene function and normal and pathological pathways in cartilage. In addition, these disorders revealed genetic mechanisms such as allelic, locus and clinical heterogeneity which often disguise a basic Mendelian inheritance. For example, in the HGMD database of April 2006, 112 different mutations in the *COL2A1* gene have been described resulting in 16 different rare skeletal dysplasias. Alternatively, families have been described with rare early onset OA in the absence of chondrodysplasia for which several genes responsible for osteochondrodysplasias have been excluded[50-52]. Identification of mutations in these families might be instructive to understand polygenic inheritance of OA and may provide insights into the molecular processes that lead to articular cartilage destruction in a wider range of OA patients than just those with skeletal dysplasias.

1.5 Genetic approaches

The applied approaches to discover genes for complex diseases such as OA fall broadly into two categories: hypothesis-based candidate gene approaches mainly on the basis of association studies and exploratory genome-wide approaches based on both linkage and genome-wide association studies.

1.5.1 Candidate gene studies by genetic association

The fundamental requirements for a candidate-gene approach by genetic association are: a candidate gene that may be involved in the OA process, one or more polymorphic variants within the gene and a suitable population consisting of cases and controls. Candidate genes are usually selected on the basis of functional or positional information. Functional candidate genes are selected on a priori made hypothesis if their protein products seem relevant to the pathogenesis of the disease, such as genes encoding for collagen type II, IX and XI. Genes might be selected on the basis of a differential RNA expression pattern in OA cartilage as compared to healthy cartilage. Alternatively, positional candidate genes for OA are selected because they lie within linkage regions identified by genome-wide scans.

Genetic association studies test whether a variant in a candidate gene is correlated to a phenotype or trait on a population scale. An association between an allele and OA might arise by several mechanisms depending on the variant and population studied. First, the allele itself has a functional effect (direct association). Second, the allele is in linkage disequilibrium (LD) or correlated with a nearby functional allele (indirect association). Third, the association is a false positive e.g. due to confounding

or by selection bias[53]. Most of the genome falls into long segments of strong LD with low haplotype diversity (haplotype blocks)[54-56]. Since SNPs in these haplotype blocks are strongly correlated with each other and often redundant, one variant (tagging SNP) can serve as a proxy for many others in an association study. In such indirect association approaches, a subset of tagging SNPs can be chosen to capture most of the underlying common variation[54;57].

In a case-control study, we test whether a genetic variant is more common in cases than controls. This is the most widely applied design for detecting associations. This approach has several important advantages: the methodology is well-understood and easy to perform, has large power to find modest genetic factors[58;59], efficient recruitment of large sample sizes of cases and controls, possibility to study late-onset disease and are often used for replication of previous findings[53;60]. Despite its ease, however, genetic association studies often fail to replicate the original association in subsequent studies[61-63]. The lack of reproducibility is generally ascribed to population stratification, insufficient statistical power, phenotypic heterogeneity and population-specific LD[53;59;60;64].

1.5.2 Genome-wide approaches

Genome-wide approaches can be applied to detect a region in the genome at which evidence accumulates for the presence of genes that may contribute to the disease. Positional information reduces the list of possible candidates from all 30.000 to maybe 10-100 genes. Linkage analysis methods tests for cosegregation of a chromosomal region and a trait of interest and can be applied to both Mendelian disorders (parametric linkage) and complex diseases (non-parametric linkage). Parametric linkage analysis requires specification of the inheritance model and tests cosegregation of genetic loci in pedigrees in which OA segregates as Mendelian trait and have large power to identify a significant region of linkage. Difficulties, however, are introduced by heterogeneity, complexity of the mode of inheritance and misdiagnosis, admixture of sporadic cases which could lead to a wrong inheritance model that may lead to a false negative signal[59].

Non-parametric linkage analyses, is not influenced by allelic heterogeneity and do not require a specification of the disease model. It tests for excessive sharing of alleles that are identical by descent (IBD) among affected relatives often affected siblings. According to the null hypothesis of no linkage, the number of IBD alleles shared in siblings is none with probability 0.25, one with probability 0.50 or two with probability 0.25. Linkage is observed if the affected sibling pairs share significantly more alleles IBD at a locus than expected under the null hypothesis. Linkage analysis has mainly been successful for mapping genes that underlie Mendelian disorders.

For most common diseases, linkage analysis has achieved only limited success[65;66]. First, the genes discovered usually explain only a small fraction of the overall heritability of the disease. Second, a large genetic component and a large number of sibling pairs are necessary to identify genetic loci. Third, the linkage regions are generally broad which makes it difficult to pinpoint causal variants. Additional approaches including fine mapping and positional cloning of genes in the linkage region are required to map the causal gene[65].

New developments and insights in complex diseases including the publication of a first draft of the sequence of the human genome[67;68], the development of high-throughput genotyping technologies, millions of single nucleotide polymorphisms (SNPs) into public databases, the International HapMap Consortium[69] and collection of large-scale OA cohorts[70] have facilitated the identification of OA genes. These advances have led to alternative gene mapping approaches such as genome-wide association studies and/or LD mapping of linkage regions in which a dense set of SNPs across the genome or region is genotyped[65].

1.6 Genetic studies in osteoarthritis

In early OA studies, candidate genes such as the vitamin D receptor (VDR)[71-83] and oestrogen receptor α (ESR1)[84-87] have received considerable attention. Some of these studies demonstrated positive associations while others failed to replicate the initial reports. As for other complex disease investigations, most of these studies had small sample sizes and were focused on small numbers of polymorphisms in single genes ignoring genetic interactions and LD within the genes. Over the past decade, genetic OA studies highlighted multiple susceptibility loci for OA and the first genes are emerging (Table 1). In order to interpret this table, one should realise that true replication has not been feasible due to the variety of phenotype definitions applied for the inclusion of patients in different studies. Generally, the value of genetic observations, as shown in Table 1, enhance when additional evidence in the following strategies has been observed: association, functional assays, differential expression or linkage with subsequent association.

1.6.1 Osteoarthritis phenotypes

In genetic OA studies, it is problematic to define an appropriate phenotype especially when multiple joints are involved such as for hand OA and GOA. Should hand OA be treated as quantitative trait or as qualitative trait? Which threshold of affected nodes or interphalangeal joints should be used for a case definition? Should a case

definition be based on Heberden's nodes, ROA features or ACR criteria or both? Should we analyse specific patterns of joint involvement (DIP versus CMC1)? Is spinal degeneration a part of GOA? Is development of hip OA followed by development of OA in hand or spine the same entity as hip OA only?

As an instructive example for the relevance of defining phenotypes, we discuss three genome scans on the basis of different definitions of hand OA leading to different positive linkage signals (Table 1). In families from the Framingham study, hand ROA was defined in two approaches prior to the linkage analysis of the genome scan: based on the quantity (sumscores of osteophytes, JSN, Kellgren/Lawrence scores and number of affected joints) or on clustering into specific hand joint regions with high heritabilities (DIP, PIP, CMC1, IP and MCP). In the first approach, the highest LOD scores, 2.96 and 2.86 respectively, were observed for JSN on 1p and 7p whereas the second analysis revealed even higher LOD scores for CMC1 ROA on 15q and 7q for DIP ROA (LOD score 6.25 and 3.06 respectively)[88;89]. In a scan of Icelandic subjects, clinical hand OA was analysed with a joint specific approach (DIP and/or CMC1 OA) and an OA locus on 2p (LOD score 4.97) was mapped[90]. The lack of overlapping linkage areas between the two analysis approaches within the Framingham study and the Icelandic hand OA study might reflect either that some results are false positives or that genetic heterogeneity underlies different radiographic and clinical OA features of the hand. The high heritabilities and the higher LOD scores for ROA at specific hand joints in the Framingham study indicate that part of the genetic variation may contribute to increased susceptibility for ROA at specific joints. However, some genetic variation may account for more generalised disease of the hands. These scans confirm that OA is a complex and heterogeneous disease in which multiple genes may be involved. A homogeneous subgroup of cases will be necessary to elucidate these specific genes.

1.6.2 Genes and osteoarthritis

As illustrated in Table 1, the range of genes that have been contributed to OA susceptibility so far includes genes encoding products that have either a structural function or/and a regulatory function in cartilage. Most conclusive evidence has been obtained for the genes: *IL1*, *FRZB*, *CALM1*, *ASPN* and *CILP*. These genes encode products that regulate the development and maintenance of joint tissue and mediate their effects through signal transduction pathways[107]. The hypothesis emerging from these studies is that polymorphisms in signal transduction pathways contribute to OA susceptibility in cartilage by disrupting chondrocyte-matrix associations, altering the metabolic responses in the chondrocyte and thereby disturbing cartilage homeostasis[32]. Clear classification of potential OA susceptibility genes can not be made since these fall into different functional categories simultaneously.

Table 1 Loci and genes identified in genetic OA studies.

Locus	MLS	Phenotype	Gene	Population	Approach	Ref
1p32.1-p22.1	3	hand ROA (sumscore of JSN)		296 extended pedigrees	variance components linkage analysis	88
2q24-q31.1	1.6	hip replacement	*FRZB*	378 siblings	linkage/association/functional assay	91
2q12-q21		hip ROA/Knee OA	*Il1* cluster	cohort/case-control	association/ haplotype analysis	92-96
2p24.1	4.4	DIP/CMC1 OA	*MATN3*	329 families	linkage analysis	90
2p24.2-p21	2.2	hand ROA (sumscore of JSN)		296 extended pedigrees	variance components linkage analysis	88
2q33.3-q34	4.7	early onset generalised OA		7 extended families	linkage analysis	97
3p21.31		knee (R)OA	*TNA*	cohort/case-control	expression/association analysis	98;99
3p13	1.8	DIP OA		329 families	linkage analysis	90
4q12-q21.2	3.1	hip replacement		146 siblings	linkage analysis	100
4q32.1	3.3	DIP OA		329 families	linkage analysis	90
4q35.1-q35.2		Beukes hip dysplasia		extended family	linkage analysis	52
6p12.3-q13	4	hip replacement		146 female siblings	linkage analysis	100
6q25.1		knee ROA	*ESR1*	cohorts/case-control	association analysis	84-86
7p14.1-p12.3	2.3	hand ROA (sumscore of JSN)		296 extended pedigrees	variance components linkage analysis	88
7q35-q36.1		DIP ROA		296 extended pedigrees	factor and linkage analysis	89
9q21.13-q21.33	2.3	hand ROA (sumscore of JSN)		296 extended pedigrees	variance components linkage analysis	88
9q22		knee/hip OA	*ASPN*	case-control	association/ functional assay	101
10q26		knee (R)OA	*ADAM12*	cohort/case-control	expression/association analysis	98;99
11q13.2-q14.2	1.6	hand ROA (sumscore of JSN)		296 extended pedigrees	variance components linkage analysis	88
11q13.4-q14.3	2.4;1.8	hip replacement		146 female siblings	linkage analysis	102
12q12-q14		knee (R)OA, lumbar spine	*VDR*	cohorts/case-control	association analysis	73-76;81
12q24.33	1.7	hand ROA (sumscore of JSN)		296 extended pedigrees	variance components linkage analysis	88
13q14		knee (R)OA/replacement	*LRCH1*	case-control	genome-wide association analysis	103
13q14.11-q14.3	1.6	hand ROA (sumscore of K&L)		296 extended pedigrees	variance components linkage analysis	88
14q32.11		hip OA	*CALM1*	case-control	genome-wide association analysis	104
14q32.13		knee OA	*AACT*	cohort/case-control	expression/association analysis	98;99
15q22.31		lumbar disc disease, knee OA	*CILP*	case-control	expression/association analysis	98;99;105
15q22.31-q26.1		CMC1 ROA		296 extended pedigrees	factor and linkage analysis	89
16p12.3-p12.1	1.7	hip replacement	*IL4R*	146 female siblings	linkage/association analysis	106
16p12.1	2.6	early onset hip OA		extended family	linkage analysis	51
16q22.1-q23.1	1.9	hip/ knee replacement		218 female siblings	linkage analysis	100
19q12-q13.3	1.8	hand ROA (sumscore of K&L)		296 extended pedigrees	variance components linkage analysis	88

MLS = multipoint LOD score; (R)OA = (radiographic) osteoarthritis; JSN = joint space narrowing; DIP = distal interphalangeal joint; CMC1 = first carpometacarpal joint; K&L = Kellgren & Lawrence

1.6.3 Joint development

The wingless (Wnt) signalling pathway is crucial for chondrogenesis and skeletogenesis but plays also a role in maintaining cartilage integrity[108-111]. The 2q24-31 region was mapped to families concordant for hip OA. The *FRZB* gene was located in this region encoding secreted frizzled-related protein 3, a glycoprotein that antagonises the signalling of wingless (wnt) ligands through frizzled membrane-bound receptors[112]. The R324G variant and the haplotype of both substituted arginine alleles (R200W and R324G) in *FRZB* were associated with female hip OA and showed diminished activity to antagonise the Wnt signalling *in vitro*[91]. Although the effect sizes were small and limited to female hip replacement, the combination of both genetic and functional evidence was convincing. It showed that general pathways in development, such as the Wnt signalling, may lead to susceptibility of common late-onset OA. It is possible that diminished Wnt signalling contributes to OA susceptibility also at other joint sites since patients in the UK study were not screened for OA at, for example, hand and/or spine.

1.6.4 Extracellular matrix components

Several studies have found genes encoding proteins of the ECM contributing to OA susceptibility. *MATN3* encodes the noncollagenous cartilage matrix protein matrilin-3 and presumably acts as adaptor in assembly of supramolecular structures in the ECM[113]. Following the Icelandic hand OA scan, a rare nonsynonymous conserved variant in this gene was found to be a moderate risk factor for hand OA[90]. Highly penetrant mutations in *MATN3*, mostly affecting residues of the single von Willebrand factor domain cause Multiple Epiphyseal Dysplasia[114-120]. The variant that may predispose to OA is localised in the first EGF domain of *MATN3* and may, therefore, have a milder effect. It is necessary to investigate the functionality of this variant and to confirm this association in other genetic studies.

CILP, encodes the cartilage intermediate-layer protein which acts as a regulator of TGFß signalling. This gene is upregulated in OA cartilage as compared to healthy cartilage[98]. A functional allele that inhibited TGFß signalling was associated to lumbar disc disease in the Japanese population[105]. The other allele of this polymorphism was associated with a reduced risk of knee OA in two Caucasian populations[98;99;105]. Notably, this gene is also located in one of the mapped regions for CMC1 ROA in the Framingham study and might explain this linkage signal[89].

Asporin is also an extracellular matrix protein and a member of the small leucine-rich proteoglycans (SLRP) family. It contains an aspartic acid (D) repeat of variable length known to participate in protein-protein interactions. Indeed, SLRP family members can bind growth factors (e.g. TGFß) and collagens which presumably play a role in crosslinking the collagen network with proteoglycans[121]. A compelling genetic association in two Japanese populations with knee OA and one Japanese population with hip OA of two functional alleles in the repeat of asporin was reported[101]. Subsequent functional studies showed that asporin suppressed *TGFß* mediated expression of cartilage matrix genes, with the disease associated D14 allele resulting in the greater inhibition than the D13 and other alleles[101]. Conversely, the stretch of aspartic acid residues in asporin was not associated with OA susceptibility in Caucasian populations[122-124].

1.6.5 Maintenance of cartilage matrix

Although OA is frequently regarded as a non-inflammatory arthropathy, considerable data implicate a role for cytokines in OA susceptibility[32]. Chromosome 2q12-13 harbours the interleukin-1 (*IL-1*) gene cluster encompassing *IL1a*, *IL1β* and *IL1RN*. Products of the *IL-1* cluster may contribute to cartilage loss by stimulating chondrocytes to produce cartilage matrix degrading enzymes. Initially, individual polymorphisms in the *IL-1* cluster showed specific associations to hand, hip and knee OA[92;93;96]. Haplotype analysis across the *IL-1* cluster, taking LD into account, provided convincing evidence and showed common risk haplotypes for knee OA and hip OA in independent cohorts[93;95;125].

A second member of the cytokine class associated with OA is the interleukin 4 receptor α chain (*IL4R*) on 16p. Two independent genome-wide scans with hip OA, mapped the 16p locus: an UK scan of siblings with hip replacement[126] and a 4-generational family with mainly hip OA without dysplasia[51]. Subsequent association analysis in the UK cohort showed that common nonsynonymous SNPs in interleukin *IL4R* were associated with hip OA[106]. It is unclear yet whether the affected Icelandic family members carry a mutation in the *IL4R* gene.

Through a large-scale association study, a functional allele in the calmodulin 1 (*CALM1*) gene was identified as a risk factor for hip OA in the Japanese population[104]. Calmodulin binds to intracellular Ca^{2+} and mediates various signals involved in cellular function including chondrogenic differentiation[127;128]. The associated allele reduced transcription *in vitro* and *in vivo* of the *CALM1* gene[104]. The increased OA risk therefore appeared to be acting by reducting the amount of calmodulin synthesised which then resulted in decreasing chondrogenic differentiation[104].

1.6.6 Unknown function

Through a large-scale association study (> 25,000 SNPs located in ~14,000 genes), consistent associations were observed between the *LRCH1* SNP rs912428 on chromosome 13q14 and knee OA in three samples from two populations. The precise function of the *LRCH1* gene in relation to OA risk is unknown[103]. This association reveals that unsuspected genes with unknown function may contribute to the pathogenesis of OA.

1.6.7 Summary

We conclude here that OA is a complex and clinically heterogeneous disease in which multiple genes belonging to multiple pathways may be involved. In the past decade, a number of genes have been identified by different approaches. The findings of *IL1*, *FRZB*, *CALM1*, *ASPN* and *CILP* are most convincing so far since there is additional genetic or functional evidence available. Genetic evidence was provided by replication of these associations in independent populations. Functional evidence of these findings has been obtained by functional assays or gene expression studies comparing healthy and OA cartilage.

Nevertheless, it is important to further validate these findings in additional studies to gain further insights into OA susceptibility. A striking example is the absence of associations with the functional polymorphisms in *ASPN* and *CALM1* with hip or knee OA in Caucasian populations which might be due to ethnic differences[122-124;129]. Understanding of the OA process requires, therefore, confirmations of other related phenotypes including the involvement of multiple joint sites which has received little attention so far.

1.7 Outline of this thesis

The principal aim of this thesis is to gain insights into the aetiology of OA by the identification of gene variants that contribute to the susceptibility of familial rare and common GOA and common OA in the population. In the next chapters, we have applied three different approaches to reach our aim and for each strategy another suitable population has been collected. As illustrated in figure 6, we investigated the GOA phenotype in extended Mendelian families for which only one or a few major genes are involved. Second, affected siblings (GARP study) were investigated with symptomatic OA at multiple joint sites. These siblings have a positive family history for OA and a complex mode of inheritance. Third, we investigated a sample from the Rotterdam study, a population-based study, with phenotyped cases and controls from the same source population which is appropriate to identify multiple genes of relatively small effect in association studies.

The current improvement in genetic tools and resources has facilitated the search of OA genes by high throughput genotyping which requires large-scale collection of DNA with high quality appropriate for genotyping with high success rate. In chapter 2, the yield and genotyping success rate of mouth swab samples extracted from buccal cells will be discussed.

Current genetic studies focus on OA at a single joint. Many people with OA in one joint may also have the disease in other joints. Therefore, in the ensuing chapters of this thesis, we focus on the localisation and identification of genetic susceptibility loci for OA at multiple joint sites. In chapter 3, we investigated whether previously

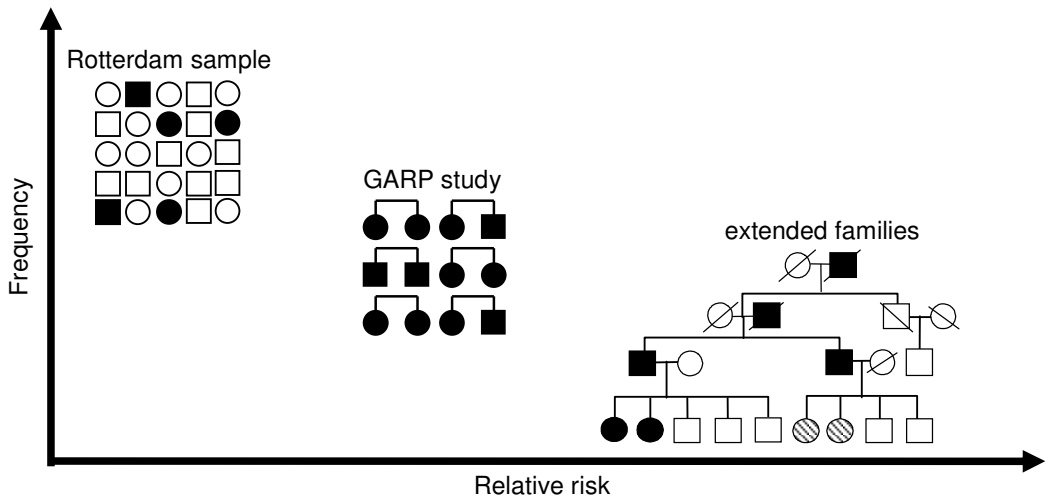

Figure 6 Difference in relative risk of disease causation between single variants with major effect in early onset families and multiple common variants in the random population with each a low effect size.

reported associations in specific joint sites can be extended to other heritable more generalised phenotypes. For these studies, we used a random sample from the population-based Rotterdam study, comprising either 1,369 subjects aged 55-70 years (chapter 3.1) or 809 subjects aged 55-65 years (chapter 3.2) which are scored for ROA signs in hand, hip, and knee and spinal disc degeneration. The studies include two variants in the *FRZB* gene previously associated to hip OA (Chapter 3.1) and a rare variant in the *MATN3* gene previously associated to hand OA (Chapter 3.2).

Chapter 4 en 5 present results of two different genome-wide scans which are performed to map chromosomal regions harbouring loci contributing to the early and late onset of OA at multiple joint sites. Chapter 4 describes the 10 cM genome-wide scan in 191 siblings from the GARP study with clinical OA at multiple joint sites. First, it was investigated whether loci could be detected by nonparametric linkage analysis. Second, fine mapping of the loci was performed by typing additional markers. Third, tagging SNPs in candidate genes were genotyped and simultaneous analysis of linkage and association was performed to investigate to what extent the genotyped SNPs contribute to the observed linkage signal. Chapter 5 presents linkage analysis of seven large extended families and mutation analysis of the positional candidate genes *PTHR2* and *FZD5* (Chapter 5.1) and the extended mutation analysis of 18 other positional candidate genes (Chapter 5.2). In chapter 6, the results and future perspectives are discussed.

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