Genetic epidemiology of osteoarthritis

Studies of familial aggregation and candidate genes

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Onderzoek naar familie aggregatie en kandidaat genen

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Chapter 4

Bijkerk C, Meulenbelt I, Odding E, Valkenburg HA, Miedema HS, Breedveld FC, Hofman A, te Koppele JM, Pols HAP, van Duijn CM, Slagboom PE. Association of the COL2A1 gene with radiological osteoarthritis in a population-based study. The Rotterdam Study. Submitted.

Bijkerk C, Meulenbelt I, de Wildt SC, Pols HAP, Breedveld FC, Hofman A, te Koppele JM, van Duijn CM, Slagboom PE. The COL9A1 gene and COL11A2 region and radiographically assessed osteoarthritis in subjects from a population-based study. Submitted.

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Bijkerk C, Meulenbelt I, Hofman A, Breedveld FC, Pols HAP, van Duijn CM, Slagboom PE. Interaction between the IGF-1 and COL2A1 genes in the association with radiological osteoarthritis. Submitted.

Other publications by the author

Meulenbelt I, Bijkerk C, Breedveld FC, Slagboom PE. Genetic linkage analysis of 14 candidate gene loci in a family with autosomal dominant osteoarthritis without dysplasia. J Med Genet 1997;34:1024-7.

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1

Introduction

Osteoarthritis (OA) is the most common rheumatic disease and an important cause of disability in the elderly (1,2). It is characterized by a progressive degeneration of articular cartilage of diarthrodial joints without synovial inflammation or bone erosions. It leads in a minority of subjects to clinical OA, i.e. joint pain, limited range of motion of the affected joint, joint effusion, local inflammatory reaction or crepitus. The clinical diagnosis of OA is confirmed by radiographic evidence, reflecting deterioration of cartilage with narrowing of joint space, formation of osteophytes at the joint margins, development of sclerosis of subchondral bone and development of pseudocystic areas in subchondral bone.

OA is a chronic disease with a multifactorial etiology that includes genetic factors (e.g. skeletal disorders, heritable forms of obesity), other systemic factors (e.g. age, sex, race, bone mineral density), biomechanical factors (e.g. trauma, joint deformity, muscle weakness) and environmental factors (e.g. nutrition, sports, estrogen replacement therapy). The genetic influence on the etiology of OA has long been recognized for women with Heberden's nodes and for patients with generalized OA (3,4). There is growing evidence from population-based studies, that common forms of OA, such as hand and knee OA, are also heritable (5-7). Various mutations in several genes have been detected in families with severe early-onset OA associated with heritable disorders as osteochondrodysplasia, Stickler syndrome, chondrocalcinosis or epiphyseal dysplasia (8,9). It remains largely unclear which genes are involved in causing common forms of OA that occur in an elderly population. Finally, genetic susceptibility to OA could also result from genetic influences on risk factors for OA, like obesity and increased bone mineral density.

This thesis first describes some issues of consideration when studying the genetic epidemiology of a complex disease as osteoarthritis (Chapter 2.1). Next, the methods of the studies presented in this thesis are described. Radiological OA (ROA) in knees, hips, and hands and disk degeneration of the spine was assessed in a large population-based sample (Chapter 2.2) and in a selected sample of siblings (Chapter 2.3). Subsequently, four main issues in the genetic epidemiology of OA are addressed. Firstly, the clustering of OA at multiple joint sites is examined and risk factors for generalized OA are studied (Chapter 3.1). Secondly, in a sibling pair study, the familial aggregation of knee, hip, and hand ROA, of disk degeneration of the spine, and of the combined presence of ROA and disk degeneration at multiple sites was examined (Chapter 3.2). Thirdly, three collagen genes were studied as candidate genes for OA, as they encode structural proteins of articular cartilage, in an association and a sibling pair study (Chapter 4). Fourthly, the role of a non-collagen candidate gene, the insulin-like growth factor-1 (IGF-1) gene, was examined, together with the possible interaction with the procollagen type II (COL2A1) gene (Chapters 5.1 and 5.2).

Finally, a general discussion concerning the validity and implications of the reported results is given in Chapter 6, together with suggestions for future research.

References

1. Masi AT, Medsger TA. Epidemiology of the rheumatic diseases. In, Arthritis and allied conditions. McCarthy DJ (ed). London. Lea and Febiger. 1989;16-54.
2. Odding E, Valkenburg HA, Algra D, van den Ouweland FA, Grobbee DE, Hofman A. Associations of radiological osteoarthritis of the hip and knee with locomotor disability in the Rotterdam Study. Ann Rheum Dis 1998;57:203-8.
3. Stecher RM. Heberden's nodes. Heredity in hypertrophic arthritis of the finger joints. Am J Med Science 1941;201:801.
4. Kellgren JH, Lawrence JS, Bier F. Genetic factors in generalized osteoarthrosis. Ann Rheum Dis 1963;22:237-55.
5. Spector TD, Cicuttini F, Baker J, Loughlin J, Hart D. Genetic influences on osteoarthritis in women: a twin study. BMJ 1996;312:940-3.
6. Felson DT, Couropmitree NN, Chaisson CE, Hannan MT, Zhang Y, McAlindon TE, LaValley M, Levy D, Myers RH. Evidence for a mendelian gene in a segregation analysis of generalized radiographic osteoarthritis. Arthritis Rheum 1998;41:1064-71.
7. Hirsch R, Lethbridge-Cejku M, Hanson R, Scott WW, Reichle R, Plato CC, Tobin JD, Hochberg MC. Familial aggregation of osteoarthritis. Arthritis Rheum 1998;41:1227-32.
8. Spranger J, Winterpacht A, Zabel B. The type II collagenopathies: a spectrum of chondrodysplasias. Eur J Pediatr 1994;151:407-15.
9. Vikkula M, Mariman ECM, Lui VC, Zhidkova N, Tiller G, Goldring M, van Beersum S, de Waal Malefijt M, van den Hoogen F, Ropers HH, et al. Autosomal dominant and recessive osteochondrodysplasias associated with the COL11A2 locus. Cell 1995;80:431-7.

2

Methods

Methodological considerations concerning the genetic epidemiology of osteoarthritis

Introduction

In recent years, considerable progress has been made in unraveling the etiology of several important monogenetic heritable diseases such as Huntington's disease and cystic fibrosis (1,2). In these disorders there is a direct causal relationship between the genetic defect and the occurrence of disease. However, a growing number of studies at present are focused on complex disorders. These studies have become possible through the availability of saturated marker maps of the human genome, improved statistical methods for genetic studies, and high throughput genotyping technology (3, 4). Characteristic for these complex diseases is that multiple genetic factors may play a role in their etiology. With the growing attention directed towards complex disorders in studies concerning genetic factors, the methodology of these studies and the analysis of the acquired data have changed dramatically. In this chapter the methodological difficulties in the genetic epidemiology of complex disorders will be discussed, in light of our studies on osteoarthritis (OA).

OA is a heritable disease, with a substantial influence of genetic factors in the occurrence of hand and knee ROA in the general population (sib-sib correlations range for hand ROA from 0.24 to 0.65 and for knee ROA from 0.06 to 0.17) (5-7). Which genes are involved in the development of common forms of OA is not clear at the moment. A number of genes were identified as being associated with the occurrence of OA, e.g. the procollagen type II (COL2A1) gene

(8), and the genes encoding the cartilage matrix protein (CRTM) and the cartilage link protein (CRTL1) (9). However, these associations need to be confirmed and further investigated. Next to multiple systemic and environmental factors that have been identified as risk factors for OA, e.g. age, sex, race, and nutrition, some other risk factors for OA are also genetically determined, e.g. body mass index and bone mineral density (10). Moreover, interaction probably exists between genetic factors and environmental factors in causing OA or in influencing the progression of OA.

Methodology in genetic epidemiological research of osteoarthritis

Linkage studies

In families, segregation of genetic markers can be studied through linkage analysis, a likelihood method that correlates the segregation of a disease with that of well-localized polymorphic markers (11,12). If the alleles from two loci tend to be transmitted together to offspring they are considered linked and located close to each other on the same chromosome. The genetic distance between two loci can be expressed as the recombination rate and two loci at a distance of less than 50% recombination are considered genetically linked. The basics of linkage analysis is to test the hypothesis that a polymorphic locus is linked to a disease locus at a recombination rate smaller than 50%, i.e. that the polymorphic locus is transmitted more often to affected subjects than expected by chance.

Family studies have contributed substantially to the detection of heritable causes in human disorders. Linkage studies within families in whom the disease segregates according to a Mendelian pattern, e.g. dominant or recessive, have also played a major role in the detection of the candidate genes that are involved in OA. More than 20 different mutations in the COL2A1 gene have been described, mainly leading to severe early-onset OA associated with osteochondrodysplasias (13). Also, a few mutations in the COL2A1 gene have been reported that cause familial generalized OA with relatively mild chondrodysplasia (14) or without signs of dysplasia (15). Several other genes, including the COL9A1, COL11A1, COL11A2 genes, have been identified as candidate genes for OA partly through linkage studies in families and partly through findings in studies using animal models (16-18). The pedigrees with familial generalized OA, in whom a mutation was detected, represent only a small part of the OA occurring in the population. In fact, only for a minority of families OA could be attributed to a mutation in one of the known candidate genes (15).

In case of late-onset forms of OA, large pedigrees extending over several generations are seldom at hand due to normal mortality in the elderly. Classical linkage studies are based on the assumption that one gene or at most a few genes play a role in the etiology of a disease within the families studied (19). Further, it is assumed that the inheritance pattern is known within affected families. For late-onset forms of OA, multiple genes and environmental factors are involved in the etiology. As a consequence, the statistical power of classical linkage studies for such a complex trait is low and should therefore be regarded of limited value.

Sibling pair studies

The sibling pair study is an alternative method of research within the concept of family studies. Sibling pairs for studies on late-onset disorders are in general easier to recruit than extensive pedigrees with affected family members. Different strategies are available in sibling pair studies. Firstly, recruitment of siblings that are both affected with OA according to preset criteria, e.g. knee OA absent or present, meaning the disorder is regarded a qualitative trait. Secondly, an approach in which the disorder is treated as a quantitative trait. In case of OA, the sum of the total number of joints affected with ROA could be regarded a quantitative trait. In this approach, all available siblings in a family contribute to the analyses.

The affected sibling pair method uses the concept of allele sharing to determine whether a certain locus plays a role in the occurrence of the disease under study. The hypothesis is that two siblings, who are both affected with OA, share more of their genetic information for the locus or loci that are involved in the etiology of OA than according to chance. In the quantitative trait locus (QTL) approach, the assumption is that for loci determining the trait, the variance between siblings decreases as they share alleles of a marker at that locus. To consider OA a quantitative trait is a relatively new concept (6,7). This concept is based on radiological data derived from multiple joints, e.g. hands, knees, hips, feet, and spine.

The number of sibling pairs that is required to detect a causal gene in the case of a complex disorder is large, i.e. estimates range from 250 to over 800 sibling pairs (20). Many studies up to date have been conducted with a considerably smaller number of affected sibling pairs, which has had serious repercussions for their reproducibility. For OA, only one sibling pair study of limited size (n = 76) has been published, concerning the genes COL2A1, CRTM, and CRTL-1 (21). No excess allele sharing was found in affected sibling pairs. Recently, a study has been reported showing no involvement of the COL2A1 and Vitamin D receptor (VDR) loci in a sibling pair study on hand ROA and knee ROA (22). Two other recent reports, concerning genomic screens in respectively sibships

with ROA from the genetically isolated Finnish population, and affected sibling pairs who had underwent joint replacement, showed evidence for linkage at the chromosomal regions 2q and 11q (23,24). The OA susceptibility genes in these regions remain to be determined.

Genetic association studies

Association studies are based on the assumption that linkage disequilibrium exists between a disease-susceptibility locus and a chosen marker locus, which must be present for an extensive number of meioses. In genetic association studies, unrelated individuals are studied instead of families to test candidate genes. This facilitates data collection substantially. However, in genetically heterogeneous disorders multiple mutations in different genes may exist or different mutations within one gene derived from several different founders may exist. This reduces the statistical power of genomic screens. Genetically isolated populations are more suitable for such studies, because both the number of founders and the size of these populations is limited, which has reduced the number of mutations residing in the population considerably. Which candidate genes are to be studied in a genetic association study can for example be determined by the role of the gene product in the pathophysiologic mechanism or by findings of previous studies.

The COL2A1 gene is one of the candidate genes for osteoarthritis (OA) as it encodes the α1-chain of collagen type II, which constitutes 90% of the collagen present in articular cartilage. Up to date, four association studies have focused on the COL2A1 gene with conflicting results (8,25-27). Two studies reported an association between OA and rare alleles of the COL2A1 gene (frequencies respectively 0.03 and 0.04) (8,25). Two other studies did not confirm this finding (26,27). Each of these studies was of limited size and dealt with subjects with symptomatic OA, leaving the role of the COL2A1 gene in the general population unapprised. Other association studies found evidence for an association between different subgroups of OA and respectively the CRTM and CRTL1 genes (9), the human aggrecan gene (AGN) (28), the alpha 1-antichymotrypsin gene (A1ACT) (29), and the Vitamin D receptor gene (30,31).

Validity in genetic epidemiological studies

External validity

Genetic research concerning complex disorders is prone to bias. A small sample

size can lead to a false negative result. The use of less stringent criteria for appliance of a significance level can lead to false positive conclusions. Repetitive testing with different markers could give rise to false positive results in both family studies and association studies. In general, a false positive result will eventually be recognized as false, whereas a false negative result could remain unrecognized. Genomic screening is necessary when no or little knowledge is available about putative risk or susceptibility loci of a disease. In a genomic screen 300-1000 marker loci may be tested. When multiple marker loci or marker loci with multiple alleles are used, it is sensible to apply a much higher level of statistical significance than the normal p-value of 0.05. However, what level of significance should be applied is an issue of debate. Lander and Kruglyak suggested that the significance level should be corrected for the total number of possible marker loci tested, i.e. an infinite number (32). This renders very conservative levels of significance, probably giving rise to false negative results. More straightforward would be to use the actual number of marker loci used in the study to correct for multiple testing (p-value = α/ number of tests performed, in which α is the type I error, which is usually 0.05). A similar approach can be followed when examining a candidate gene. However, when assessing the risk on disease for an allele that has previously been shown to be associated with the disease, a different situation emerges in which the need for adjustment is not evident (33).

The possibility of generalizing the results of a genetic epidemiological study is determined in part by the choice of the study population. Family studies are usually conducted in highly selected populations. No bias will be present when the study aims at localizing a gene. However, the families used may not be representative for the general population. This was illustrated for OA by the finding that, although mutations in the COL2A1 gene are the most common known cause of early-onset generalized familial OA, only in about 2% of families with more common forms of generalized OA, mutations in the COL2A1 gene could be detected (15). Although findings in selected populations are biologically relevant, only an approach in a population-based sample renders results that can be generalized to other populations.

Internal validity

Misclassification

Large-scale genotyping is prone to misclassification. In family studies, these errors are usually easily recognized because of the limited possible genetic variation within families. In association studies, where only affected, not related, subjects are tested, such errors will not be detected by testing for Mendelian segregation. When misclassification is independent from the disease status, the

errors will be randomly distributed across cases and controls. This will probably lead to a dilution of the effects found and in the worst case to a false negative result. To prevent systematic errors, it is important that cases and controls are distributed randomly across gels and that reading of the gels is done blind to the disease status. Also testing for Hardy-Weinberg equilibrium (HWE) gives an indication about the probability of systematic genotyping errors.

Another issue related to misclassification in genetic epidemiological studies is linkage disequilibrium (LD) between a marker locus and a causative mutation or a linked polymorphism. The weaker the LD between marker and mutation or risk associated polymorphism, the greater the chance that a false negative association will occur. Another issue is the causal inference of a detected association. Even when a marker locus is located within the gene that is being studied, the marker locus could be in LD with another, known or unknown, gene in the vicinity. This is in particular true for association studies using a single marker locus. For OA, this issue has emerged in case of the COL2A1 gene and the Vitamin D receptor gene, at a distance of 750 kb, on chromosome 12 (30,31). The Vitamin D receptor gene encodes the receptor of the hormonally active form of Vitamin D (1,25–dihydroxyD3). This gene is associated with bone density, and bone density is associated with radiological OA (ROA), i.e. knee and hip ROA (30,31,34). Therefore, both the COL2A1 gene and the Vitamin D receptor gene are candidate genes for OA and it is difficult to determine which of both genes is causally related to ROA.

Selection bias

Selection bias may occur when the probability of being included in the study population is dependent upon the genetic factor that is being examined. An example of this is ascertainment bias in family studies. Families who are detected through an index case with a certain disease are mostly not representatives for all the patients in the population with this disease. Firstly, these families always have a positive family history, which will lead to overestimation of the familial component, which would lead to bias in a study examining the heritability of a disease in the general population. Secondly, the chance of inclusion will increase with the number of family members that are affected.

In association studies, selection bias can occur when carriership of a certain genotype of a disease gene is associated with survival, through a different rate of mortality from the disease under investigation. This type of bias makes it difficult to distinguish whether an allele is associated with the risk for disease or with disease progression.

Population stratification

This is a special form of confounding bias in association studies, also known as

admixture, that occurs when the population that is examined is a mixture of several populations with a different genetic background and differing risks of disease in the contributing populations (35). Especially in the population of the USA, where a considerable amount of admixture has occurred, this issue calls for attention. The best known example in the literature concerns a study on diabetes in Pima Indians (36). In this study the amount of admixture between Indians and Caucasians was responsible for the association that was observed between a genetic marker and diabetes. Population stratification may also occur in studies on OA. For example, primary hip OA, i.e. hip OA not caused by any known cause, is three times more frequent in white Americans than in African-Americans, whereas in Asians primary hip OA is virtually unknown (37). In case the allele frequencies of a particular marker locus are associated with ethnicity, this could give rise to a false positive association when the actual study population exists of different ethnic subgroups.

Conclusion

In search for genes involved in the etiology of late-onset OA, several study designs can be applied. Each of the designs discussed has its limitations. Therefore, confirmation of findings through different approaches is crucial. The role of classical linkage studies is limited because of the multifactorial etiology of late-onset OA and the difficulties of recruiting extended families with sufficient numbers of affected individuals. In the present thesis we aim to apply an integrated approach of population-based association studies and sibling pair studies to study common, late-onset, forms of OA. Given the lack of knowledge concerning the genetics of OA in the general population, the first aim of this thesis is to study the phenotype and heritability of late-onset OA in unbiased population series. Secondly, in these populations we will study candidate genes, including the genes COL2A1, COL9A1, COL11A2, and insulin-like growth factor-1 (IGF-1). Thirdly, a targeted genomic screen will be conducted based on the findings of previous studies. Finally, one would like to find the functional defect in a disease-susceptibility locus and understand by which mechanism the deleterious protein or its altered expression contributes to the pathogenesis of OA. Therefore, follow-up research of the genetic epidemiological findings is needed through mutation analysis and subsequent functional molecular genetic research in cell cultures, and transgenic animals. Genomic screening and functional studies are outside the scope of this thesis. The studies presented here concern the identification of the trait of interest (Chapter 3) and candidate gene studies in the general population and in sibling pairs (Chapters 4 and 5).

References

1. Baxendale S, McDonald ME, Mott R, Francis F, Lin C, Kirby SF, James M, Zehetner G, Hummerich H, Valdes J, et al. A cosmid cloning and high resolution restriction map of the 2 megabase region containing the Huntington's disease gene. Nat Genet 1994;4:181-6.
2. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, et al. Identification of the cystic fibrosis gene: Cloning and characterization of complementary DNA. Science 1989;245:1066-73.
3. McKusick V. Mapping and sequencing the human genome. N Engl J Med 1990;320:910-5.
4. Watson JD. The human genome project: past, present and future. Science 1990;248:44-91.
5. Spector TD, Cicuttini F, Baker J, Loughlin J, Hart D. Genetic influences on osteoarthritis in women: a twin study. BMJ 1996;312:940-3.
6. Felson DT, Couropmitree NN, Chaisson CE, Hannan MT, Zhang Y, McAlindon TE, LaValley M, Levy D, Myers RH. Evidence for a mendelian gene in a segregation analysis of generalized radiographic osteoarthritis. Arthritis Rheum 1998;41:1064-71.
7. Hirsch R, Lethbridge-Cejku M, Hanson R, Scott WW, Reichle R, Plato CC, Tobin JD, Hochberg MC. Familial aggregation of osteoarthritis. Arthritis Rheum 1998;41:1227-32.
8. Hull R, Pope FM. Osteoarthritis and cartilage collagen genes. Lancet 1989;i:1337-8.
9. Meulenbelt I, Bijkerk C, de Wildt SC, Miedema HS, Valkenburg HA, Breedveld FC, Pols HAP, te Koppele JM, Sloos VF, Hofman A, Slagboom PE, van Duijn CM. Investigation of the association of the CRTM and CRTL1 genes with radiographically evident osteoarthritis in subjects from the Rotterdam Study. Arthritis Rheum 1997;40:1760-5.
10. Cerhan JR, Wallace RB, El-Khoury GY, Moore TE. Risk factors for progression to new sites of radiographically defined osteoarthritis in women. J Rheumatol 1996;23:1565-78.
11. Lander ES. Mapping complez genetic traits in humans. In: Davies KE (ed), Genome analysis: a practical approach. Oxford: IRL Press, 1988;171-89.
12. Ott J. Analysis of human genetic linkage: Revised edition. Baltimore & London: The John Hopkins University Press, 1991.
13. Prockop DJ, Kuivaniemi H, Tromp G. Heritable disorders of connective tissue. In, Harrison's principles of internal medicine, thirteenth edition. Wilson JD, Braunwald E, Isselbacher KJ, Petersdorf RG, Martin JB, Fauci AS, Root RK, editors. New York, McGraw-Hill. 1993.
14. Pun YL, Moskowitz RW, Lie S, Sundstrom WR, Block SR, McEwen C, Williams HJ, Bleasel JF, Holderbaum D, Haqqi TM. Clinical correlations of osteoarthritis associated with a single-base mutation (Arginine^{519} to Cysteine) in type II procollagen gene. Arthritis Rheum 1994;37:264-9.
15. Ritvaniemi P, Körkkö J, Bonaventure J, Vikkula M, Hyland J, Paassilta P, Kaitila I, Kaariainen H, Sokolov BP, Hakala M. Identification of COL2A1 gene mutations in patients with chondrodysplasias and familial osteoarthritis. Arthritis Rheum 1995;38:999-1004.
16. Fassler R, Schnegelsberg P, Dausman J, Shinya T, Muragaki Y, McCarthy MT, Olsen BR. Mice lacking α1(IX) collagen develop non-inflammatory degenerative joint disease. Proc Natl Acad Sci USA 1994;91:5070-4.
17. Richard AJ, Yates JRW, Williams R, Payne SJ, Pope FM, Scott JD, Snead MP. A family with Stickler syndrome type 2 has a mutation in the COL11A1 gene resulting in the substitution of glycine 97 by valine in α1(XI) collagen. Hum Mol Genet 1996;5:1339-43.

18. Brunner HG, Beersum SEC, Warman ML, Olsen BR, Ropers HH, Mariman ECM. A Stickler syndrome gene is linked to chromosome 6 near the COL11A2 gene. Hum Mol Genet 1994;3:1561-4.
19. Khoury MJ, Beaty TH, Cohen BH. Fundamentals of genetic epidemiology. New York & Oxford, Oxford University Press, 1993;164-99.
20. Fulker DW, Cardon LR. A sib-pair approach to interval mapping of quantitative trait loci. Am J Hum Genet 1994;54:1092-103.
21. Loughlin J, Irven C, Fergusson C, Sykes B. Sibling pair analysis shows no linkage of generalized osteoarthritis to the loci encoding type II collagen, cartilage link protein or cartilage matrix protein. Br J Rheumatol 1994;33:1103-6.
22. Baldwin CT, Joost O, Chaisson C, McAlindon T, Farrer L, Ordovas J, Schaefer E, Levy D, Myers R, Felson D. The type II collagen/Vitamin D receptor locus and osteoarthritis: The Framingham Osteoarthritis Study. Arthritis Rheum 1998;41:S352.
23. Leppävuori JK, Kujala UM, Kaprio J, Nissilä M, Heliövaara M, Kinnunen J, Koskenvuo M. Genome scan for predisposing loci of distal interphalangeal joint osteoarthritis. Am J Hum Genet 1998;63:A297.
24. Chapman K, Mustafa Z, Irven C, Smith A, Carr A, Clipsham K, Chitnavis J, Sinsheimer J, Sykes B, Loughlin J. A comprehensive genome-wide screen for susceptibility loci in osteoarthritis: linkage to chromosomes 2q and 11q. Am J Hum Genet 1998;63:A16.
25. Loughlin J, Irven C, Athanasou N, et al. Differential allelic expression of the type II collagen gene (COL2A1) in osteoarthritic cartilage. Am J Hum Genet 1995;56:1186-93.
26. Vikkula M, Nissilä M, Hirvensalo E, et al. Multiallelic polymorphism of the cartilage collagen gene: no association with osteoarthritis. Ann Rheum Dis 1993;52:762-4.
27. Aerssens J, Dequeker J, Peeters J, Breemans S, Boonen S. Lack of association between osteoarthritis of the hip and gene polymorphisms of VDR, COL1A1, COL2A1 in postmenopausal women. Arthritis Rheum 1998;41:1946-50.
28. Horton W, Balakir R, Precht P, Plato CC, Tobin JD, Lethbridge-Cejku M, Hochberg MC, Meek L, Doege K. Association between an aggrecan polymorphic allele and bilateral hand osteoarthritis in elderly white men. Arthritis Rheum 1996;39(Suppl):S167.
29. Sakkas LI, Macfarlane DG, Bird H, Welsh KI, Panayi GS. Association of osteoarthritis with homozygosity for a 5.8 kb Taq I fragment of the alpha 1-chymotrypsin gene. Br J Rheumatol 1990;29:245-8.
30. Uitterlinden AG, Burger H, Huang Q, Odding E, van Duijn CM, Hofman A, Birkenäger JC, van Leeuwen JPTM, Pols HAP. Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J Clin Invest 1997;100:259-63.
31. Keen RW, Hart DJ, Lanchbury JS, Spector TD. Association of early osteoarthritis of the knee with a Taq 1 polymorphism of the Vitamin D receptor gene. Arthritis Rheum 1997;40:1444-49.
32. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 1995;11:241-7.
33. Rothman KJ. Causes. Am J Epidemiol 1976;104:587-92.
34. Eisman JA. Vitamin D Receptor gene alleles and osteoporosis: an affirmative view. J Bone Miner Res 1995;10:1289-93.
35. Lander ES, Schork NJ. Genetic dissection in complex traits. Science 1994;265:2037-48.
36. Knowler WC, Williams RC, Pettitt DJ, Steinberg AG. Gm 3;5,13,14 and type 2 diabetes mellitus: an association in American Indians with genetic admixture. Am J Hum Genet 1988;43:520-6.
37. Hoaglund FT, Oishi CS, Gialamis GG. Extreme variations in racial rates of total hip arhtroplasty for primary coxarthrosis: a population-based study in San Francisco. Ann Rheum Dis 1995;54:107-10.

Assessment of radiological osteoarthritis in peripheral joints and of disk degeneration of the spine

A population-based sample aged 55 to 70 years

Subjects

To estimate the prevalence of radiological osteoarthritis (ROA) and disk degeneration of the spine in the general population, a random sample was drawn from the Rotterdam Study. The Rotterdam Study is a prospective population-based cohort study of determinants and prognosis of chronic diseases in the elderly (1). For this study, all inhabitants of a suburb of Rotterdam, aged 55 years and over, including institutionalized persons were invited to participate. In total 7983 participants (response rate 78 percent) were examined for the first time at the research center between 1990 and 1993.

We focused in this thesis on ROA and disk degeneration of relatively early-onset (55 to 70 years), as in the early-onset forms of ROA genetic influences are expected to be more prominent. Late-onset ROA is more likely the result of aging and/or the accumulation of environmental influences on ROA. Institutionalized persons were excluded (n = 16), because no radiographs were available. Of the non-institutionalized persons below 70 years of age (n = 3908, 1713 men and 2195 women) radiographs of the knees and hips had previously been scored for ROA in 1701 individuals (43.3 percent). This was performed as part of studies concerning the association between ROA and locomotor disability in the elderly (2). For the present thesis, available radiographs of the hands and spine were scored for respectively ROA and disk degeneration in these subjects. Of the 1701

individuals, radiographs could be tracked down for 1583 persons, 666 men and 917 women. In Table 1, baseline characteristics, including the major risk factors for OA (age, body mass index, and bone mineral density), for all 3908 non-institutionalized individuals below 70 years of age of the Rotterdam Study and the sample of 1583 individuals, derived from the total sample of 3908, used in the present thesis are shown.

Radiographic measurements

Weightbearing anterior-posterior pelvic radiographs with both feet in 10° endorotation, weightbearing anterior-posterior knee radiographs with the patellae in central position, posteroanterior radiographs of both hands and three lateral radiographs of the thoracolumbar spine were obtained. Radiographic data was complete for 1542 individuals (97.4 percent). Radiographs of the knees and hips were in both instances for 15 persons not available. Lateral radiographs of the thoracic, lumbar and lumbosacral spine were missing for respectively 15, 13 and 27 individuals. At the start of the Rotterdam Study only radiographs of the right hand were made, causing that radiographic data of the left hand was missing for 50 persons.

ROA in the knees, hips, and hands was assessed by means of the Kellgren grading system in five grades (0-4) using the figures and legends of the original atlas (3). Definite ROA at a particular joint site is defined as a Kellgren-score two or over. The definition of grades in the Kellgren grading system is different for the hip joints as compared to the knee and hand joints, as is outlined in Table 2. Both osteophytes and joint space narrowing need to be present in a hip joint for a Kellgren-score of two or higher, whereas in the knee and hand osteophytes accompanied by possible joint space narrowing is sufficient (see Table 2). Disk degeneration of the spine was also scored using the Kellgren grading system, based on the definitions outlined in Table 3).

Two independent readers, blinded to all other data of the participant scored all radiographs. After each set of 100-150 radiographs the scores of the two readers were evaluated. Whenever the scores were two or more points different, or, was two for one reader but one for the other, a consensus score was agreed upon. For the knees only the tibiofemoral joint could be assessed. ROA of the hand was assessed in the distal interphalangeal (DIP) joints, the interphalangeal joint of the thumb (IP), the proximal interphalangeal (PIP) joints, the metacarpalphalangeal (MCP) joints, the first carpometacarpal (CMC 1) joints, the trapezoscaphoideal (TS) joints, the radionavicular (RN) joints and the distal radioulnar (RU) joints. Disk degeneration of the spine was assessed at three levels,

Table 1
Baseline characteristics of the Rottedam Study and randomly drawn study population.

	The Rotterdam Study			Study population		
	Total n = 3908	Men n = 1713	Women n = 2195	Total n = 1583	Men n = 666	Women n = 917
Age[1] (SD)	62.7 (4.2)	62.9 (4.1)	62.6 (4.2)	63.1 (4.1)	63.4 (4.1)	62.9 (4.1)
BMI[2] (SD)	26.2 (4.0)	25.8 (4.0)	26.6 (4.0)	26.3 (3.7)	25.9 (3.0)	26.6 (4.2)
BMD[3] (SD)	0.86 (0.13)	0.89 (0.13)	0.84 (0.13)	0.86 (0.13)	0.89 (0.13)	0.83 (0.13)
% Heberden's nodes	17.4	12.2	21.5	21.9	15.9	26.3
Smoking[4]: % current	27.9	31.5	25.1	30.2	34.5	27.1
% former	44.2	60.6	31.3	44.1	59.3	33.0
% never	28.0	8.0	43.7	25.7	6.2	39.9
% with OA[5]	27.6	21.6	31.1	24.5	17.2	29.8
% with RA[6]	3.7	2.5	4.3	3.1	2.3	3.7

[1] Age in years.
[2] BMI = Body mass index in kg/m^2.
[3] BMD = Bone mineral density of the femoral neck in g/cm^2.
[4] Cigarette smoking.
[5] Self reported diagnosis of osteoarthritis (OA).
[6] Self reported diagnosis of rheumatoid arthritis (RA).

Table 2
Kellgren grading system for radiological osteoarthritis (ROA).

Grade		Description
Knee and Hand		
0	None	
1	Doubtful	Possible osteophytic lipping or doubtful narrowing of joint space
2	Minimal	Definite osteophytes and possible narrowing of joint space
3	Moderate	Multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends
4	Severe	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends
Hip		
0	None	
1	Doubtful	Possible osteophytes around femoral head and possible narrowing of joint space medially; or osteophytes alone
2	Minimal	Definite osteophytes, definite narrowing of joint space inferiorly and slight sclerosis
3	Moderate	Definite osteophytes, marked narrowing of joint space, some sclerosis and cyst formation and deformity of femoral head and acetabulum
4	Severe	Large osteophytes, gross loss of joint space with sclerosis and cysts, marked deformity of femoral head and acetabulum

All features are scored left and right separately. Definite ROA is defined as Kellgren-score two or over.

Table 3
Kellgren grading system for disk degeneration of the spine.

Grade		Description
0	None	
1	Doubtful	Doubtful intervertebral disk space narrowing or possible osteophytes
2	Minimal	Definite osteophytes and possible narrowing of inter-vertebral disk space
3	Moderate	Definite osteophytes, definite narrowing of intervertebral disk space and some sclerosis of vertebral endplates
4	Severe	Large ("bridging") osteophytes, marked narrowing of intervertebral disk space and sclerosis of vertebral endplates

Definite disk degeneration is defined as Kellgren-score two or over.

i.e. thoracic (Th4 to Th12), lumbar (L1 to L4 or L5) and lumbosacral (L5-S1 or L5-L6).

Prevalence of ROA and disk degeneration

The prevalence of ROA in the knees, hips, and hands and of disk degeneration of the spine in 666 men and 917 women aged 55 to 70 years is shown in respectively Tables 4 and 5. For this purpose hand ROA was defined as Kellgren-score two or over in any of the hand joints that was scored, i.e. 18 for each hand. Definite disk degeneration was defined as Kellgren-score two or over in any of the three levels that was scored.

ROA in peripheral joints, i.e. knee, hip, and hand, was clearly more prevalent in women as compared to men in all age categories, except for hip ROA before the age of 64 years. The prevalence of disk degeneration of the spine was similar for men and women. Hand ROA is almost without exception more frequent at the right-hand side than at the left-hand side. This difference is most marked in women with hand ROA. All joint sites show an increase of the percentage of affected individuals with age. In women, the increase with age was statistically significant for the hip, hand and spine ($p < 0.001$), with hip ROA showing the sharpest increase. In men, the increase of peripheral joint ROA with age leveled off and was only statistically significant for hand ROA ($p = 0.01$). Furthermore, it is important to note that at the age of 70 years more than 80 percent of women and more than 55 percent of men have one or more joints in the hands affected with ROA. The thoracolumbar spine is affected with disk degeneration at one or more levels in more than 80 percent of both men and women. This suggests that hand ROA and disk degeneration of the spine are typically aging disorders.

The number of men or women that have joint complaints together with the radiological signs of ROA or disk degeneration is considerably lower. Data on joint complaints are derived from the Rotterdam Study and apply to men and women aged 55 to 70 years. For the hands, one in three women and one in seven men have joint complaints in the hands together with hand ROA. For the hips, one in two women and one in four men have complaints in conjunction with ROA. Fifty percent of women and just 7 percent of men have both knee ROA and knee complaints.

In Table 6 the number of joint sites that is affected with ROA or disk degeneration is shown for men and women separately. At the age of 70 only 2.2 percent of women and 8.4 percent of men have not a single joint site affected with ROA or disk degeneration. The number of persons with three or more joint

Table 4
Prevalence of knee, hip, and hand ROA and of disk degeneration of the spine in 666 men aged 55-70 years from the Rotterdam Study.

	Age category (in years)					Total
	55-58	58-61	61-64	64-67	67-70	
Number with knee ROA (%)						
Left	6 (6.5)	5 (4.6)	14 (9.8)	14 (8.8)	11 (7.1)	50 (7.6)
Right	8 (8.6)	3 (2.8)	15 (10.5)	17 (10.6)	20 (12.8)	63 (9.5)
Left and/or right	10 (10.8)	7 (6.4)	23 (16.1)	24 (15.1)	23 (14.7)	87 (13.2)
Number with hip ROA (%)						
Left	6 (6.5)	8 (7.3)	14 (9.9)	14 (8.7)	8 (5.1)	50 (7.6)
Right	6 (6.5)	10 (9.2)	17 (12.0)	15 (9.3)	15 (9.6)	63 (9.5)
Left and/or right	8 (8.7)	12 (11.0)	19 (13.4)	20 (12.4)	20 (12.7)	79 (12.0)
Number with hand ROA (%)						
Left	22 (23.4)	29 (26.4)	49 (34.0)	71 (44.1)	67 (42.7)	238 (35.7)
Right	25 (26.6)	38 (34.5)	58 (40.3)	75 (46.6)	71 (45.2)	267 (40.1)
Left and/or right	35 (37.2)	42 (38.2)	71 (49.3)	89 (55.3)	89 (56.7)	326 (48.9)
Number with disk degeneration of the spine (%)	55 (59.8)	61 (56.0)	102 (72.3)	116 (73.0)	125 (80.1)	459 (69.9)

Numbers listed are number of individuals affected, with in brackets the percentage within the according age category. Definite ROA or disk degeneration was defined as Kellgren-score two or over.

Table 5
Prevalence of knee, hip, and hand ROA and of disk degeneration of the spine in 917 women aged 55-70 years from the Rotterdam Study.

	Age category (in years)					Total
	55-58	58-61	61-64	64-67	67-70	
Number with knee ROA (%)						
Left	12 (8.3)	21 (11.8)	34 (16.6)	29 (14.6)	27 (14.8)	123 (13.5)
Right	15 (10.4)	28 (15.6)	34 (16.6)	39 (19.5)	35 (19.2)	151 (16.6)
Left and/or right	20 (13.9)	34 (19.1)	47 (22.9)	47 (23.6)	43 (23.6)	191 (21.0)
Number with hip ROA (%)						
Left	3 (2.1)	9 (5.0)	6 (2.9)	17 (8.5)	19 (10.5)	54 (6.0)
Right	3 (2.1)	11 (6.2)	11 (5.4)	18 (9.0)	29 (16.0)	72 (7.9)
Left and/or right	3 (2.1)	12 (6.8)	14 (6.8)	27 (13.5)	36 (19.9)	92 (10.1)
Number with hand ROA (%)						
Left	50 (34.7)	81 (44.8)	106 (51.5)	105 (52.0)	128 (69.6)	470 (51.3)
Right	63 (43.8)	100 (55.2)	140 (68.0)	123 (60.9)	138 (75.0)	564 (61.5)
Left and/or right	75 (52.1)	115 (63.5)	150 (72.8)	137 (67.8)	153 (83.2)	630 (68.7)
Number with disk degeneration of the spine (%)	84 (58.7)	105 (59.3)	133 (66.2)	146 (73.0)	150 (81.5)	618 (68.3)

Numbers listed are number of individuals affected, with in brackets the percentage within the according age category. Definite ROA or disk degeneration was defined as Kellgren-score two or over.

sites affected with ROA and/or disk degeneration increases 2.2 times in men and 3.8 times in women between the ages of 55 and 70 years.

Earlier the prevalence of hand ROA was shown (Tables 4 and 5). In this case the hand was regarded a single joint site. In Figure 1 the prevalence of ROA at different sites in the hand is shown for men and women separately. Three interphalangeal joint sites are distinguished (DIP-, IP- and PIP-joints), next to the first carpometacarpal (CMC 1) and the trapezoscaphoideal (TS) joints. The radionavicular (RN) and distal radioulnar (RU) joints represent the joints of the wrists. In women all joint sites in the hands, except for the wrist joints, show a statistical significant increased frequency with increasing age (adjusted p-value for trend < 0.01). In men, only the IP-, the CMC 1-, the PIP-, and the MCP joints show a statistical significant increase with age (adjusted p-value for trend ≤ 0.01). The DIP-joints are most often affected with ROA in both men and women. In women the CMC 1 joint shows the strongest increase with age as compared to all other hand joints, in men this holds true for the MCP-joints. Differences between men and women in the prevalence of ROA are observed for the interphalangeal joints and the first carpometacarpal joint. The prevalences of ROA in MCP-, TS-, and wrist joints are similar in men and women.

The prevalence of disk degeneration is shown in Figure 2 for men and women separately. In none of the age categories statistically significant differ-

Table 6
Number of joint sites (knee, hip, hand, and spine) affected in 650 men and 892 women aged 55 to 70 years.

Age category	Number of joint sites affected					Total
	0	1	2	3	4	
Men						
55-58	21 (23.1)	41 (45.1)	23 (25.3)	5 (5.5)	1 (1.1)	91
58-61	26 (23.9)	50 (45.9)	27 (24.8)	6 (5.5)	0	109
61-64	20 (14.5)	46 (33.3)	55 (39.9)	17 (10.8)	0	138
64-67	15 (9.6)	57 (36.3)	67 (42.7)	17 (10.8)	1 (0.6)	157
67-70	13 (8.4)	55 (35.5)	64 (41.3)	21 (13.5)	2 (1.3)	155
Women						
55-58	30 (21.0)	56 (39.2)	46 (32.2)	10 (7.0)	1 (0.7)	143
58-61	30 (17.4)	58 (33.7)	58 (33.7)	24 (14.0)	2 (1.2)	172
61-64	20 (10.0)	58 (29.0)	92 (46.0)	27 (13.5)	3 (1.5)	200
64-67	16 (8.1)	62 (31.5)	80 (40.6)	29 (14.7)	10 (5.1)	197
67-70	4 (2.2)	40 (22.2)	84 (46.7)	42 (23.3)	10 (5.6)	180

Numbers listed are numbers of individuals affected, with in brackets the percentage within the according age category. Definite ROA or disk degeneration was defined as Kellgren-score two or over in the left and/or right corresponding joint.

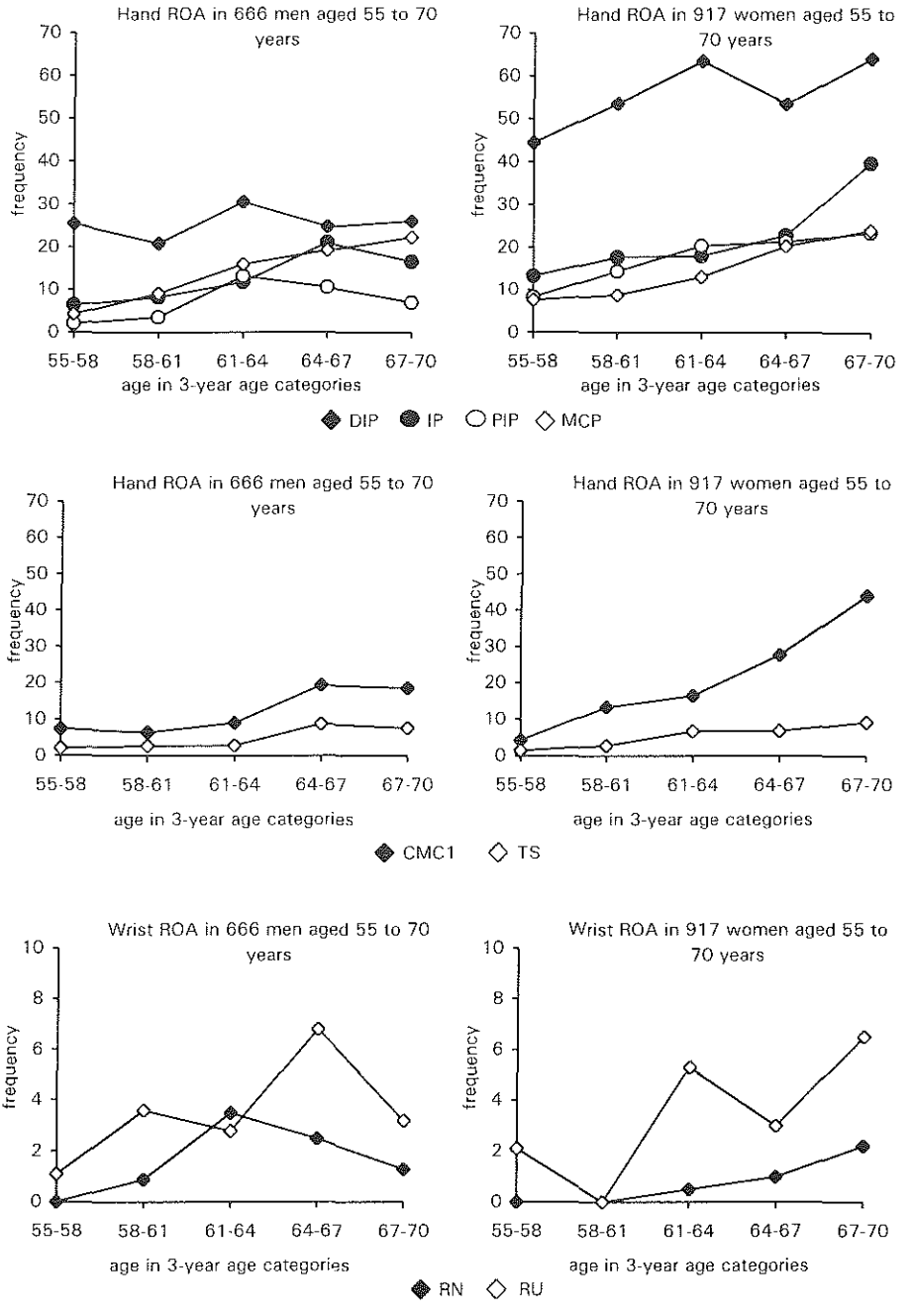


Figure 1
Prevalences of hand ROA and wrist ROA for 666 men and 917 women aged 55 to 70 years from the Rotterdam Study.

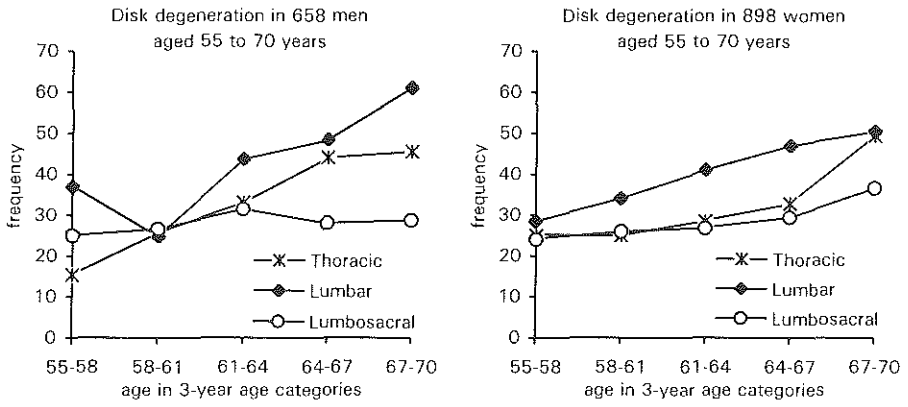


Figure 2
Prevalences of disk degeneration of the spine for 658 men and 898 women aged 55 to 70 years from the Rotterdam Study.

ences were found between men and women. A statistically significant increase with age was observed for disk degeneration of the thoracic and lumbar spine in both men and women (p-value for trend < 0.001), but neither for men nor for women for disk degeneration of the lumbosacral joint.

References

1. Hofman A, Grobbee DE, De Jong PTVM, van den Ouweland FA. Determinants of disease and disability in the elderly. Eur J Epidemiol 1991;7:403-22.
2. Odding E, Valkenburg HA, Algra D, van den Ouweland FA, Grobbee DE, Hofman A. Associations of radiological osteoarthritis of the hip and knee with locomotor disability in the Rotterdam Study. Ann Rheum Dis 1998;57:203-8.
3. Kellgren JH, Jeffrey MR, Ball J (eds). The epidemiology of chronic rheumatism. Volume II: Atlas of standard radiographs of arthritis. Oxford: Blackwell Scientific Publications, 1963.

Assessment of radiological osteoarthritis in peripheral joints and of disk degeneration of the spine

A sibling pair sample

Subjects

A sibling pair study was performed for two reasons. Firstly, to study the familial aggregation of ROA and disk degeneration and to quantify the extent to which ROA and disk degeneration are explained by genetic factors. Secondly, a sibling pair approach was used in addition to association studies, investigating the role of candidate genes in the occurrence of ROA and disk degeneration.

Probands were derived from a random sample that was drawn from the Rotterdam Study (see Chapter 2.2) and were selected based on the radiographic data present of the knees, hips, hands and spine. The following criteria were used to ascertain individuals with polyarticular ROA with a high prior probability of genetic factors playing a role in the occurrence of ROA and/or disk degeneration. Probands had to have two or more out of four joint sites affected (knee, hip, hand, and spine) and had to be between 55 and 65 years of age at the moment of their first visit to the research center between 1990 and 1993. In case individuals had hand ROA in combination with disk degeneration of the spine, the two commonest forms of ROA, Heberden's nodes had to be present additionally in order to include them as probands. Heberden's nodes are bulbous deformities at the distal interphalangeal joints of the fingers, resulting from bony outgrowths, cartilage hypertrophy or mucoid transformation of the periarticular fibroadipose tissue (1-4).

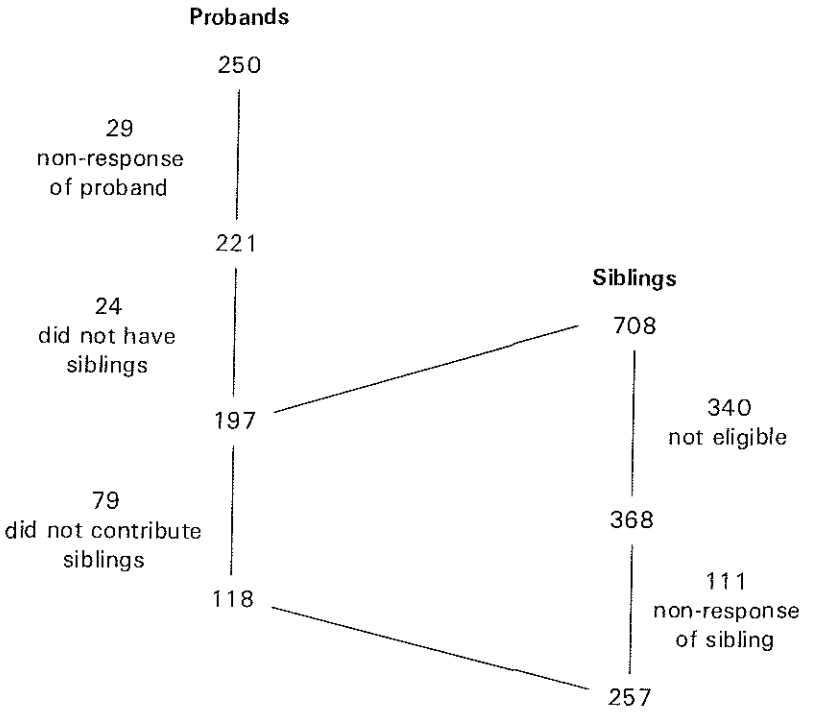


Figure 1
Participation of probands and siblings in sibling pair study.

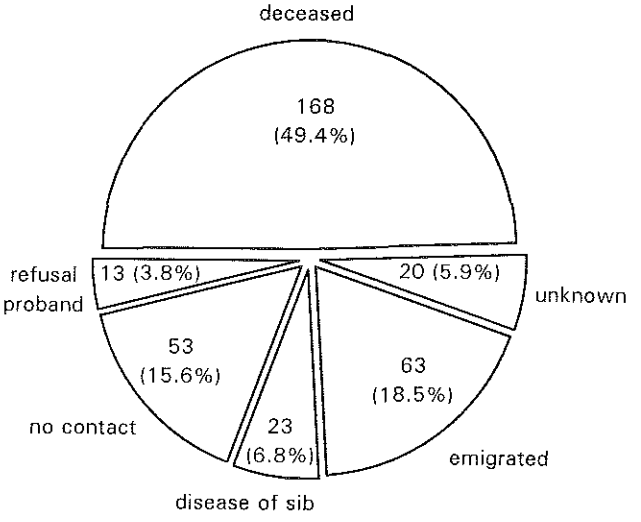


Figure 2
Reasons for non-eligibility of 340 siblings.

A flow chart outlining the participation of probands and siblings in the sibling pair study is shown in Figure 1. In total 273 persons met the criteria for inclusion, of whom at the time of the present study 10 were deceased and 13 had moved over long distance. The remaining 250 persons received a letter in which they were invited to participate in a family study concerning OA. Of the 221 probands whom agreed to take part in the study (response rate 88 percent) 24 had no siblings. The remaining 197 probands were visited at home and asked to supply the names and, if available, addresses of all their siblings born alive. The mean number of siblings per pedigree was 4.1 (including the proband), corresponding to a total of 708 siblings born alive. Of these 708 siblings, 340 were not eligible. The main reasons were death of the sibling, emigration and the absence of contact between siblings. This is shown in detail in Figure 2. The 368 siblings that were eligible also received a letter, in which they were invited to participate, which was refused by 111 siblings (response rate 70 percent). A majority (64 percent) indicated that they had no particular reason to refuse.

Thus, a total of 257 siblings, derived from 118 probands, were examined at the research center. Weight, height and blood pressure were measured according to standardized procedures. Bone mineral density was measured at the femoral neck by dual energy x-ray absorptiometry (DEXA) as described previously (5). All siblings received a questionnaire and a physical examination concerning the locomotor system, rendering information on joint complaints, loco-

Table 1
Baseline characteristics siblings.

	Total n = 257	Men n = 124	Women n = 133
Age[1] (SD)	65.3 (7.9)	64.7 (7.3)	65.8 (8.5)
BMI[2] (SD)	26.7 (4.0)	26.3 (3.0)	27.1 (4.7)
BMD[3] (SD)	0.86 (0.14)	0.88 (0.14)	0.84 (0.15)
% Heberden's nodes	29.4	23.6	34.8
Smoking[4]: % current	21.1	20.2	22.0
% former	47.9	68.1	28.5
% never	28.1	7.6	48.0
% with OA[5]	39.7	32.8	46.3
% with RA[6]	2.9	1.7	4.1

[1] Age in years.
[2] BMI = Body mass index in kg/m^2.
[3] BMD = Bone mineral density of the femoral neck in g/cm^2.
[4] Cigarette smoking.
[5] Self reported diagnosis of osteoarthritis (OA).
[6] Self reported diagnosis of rheumatoid arthritis (RA).

Table 2
Prevalence of knee, hip, and hand ROA and of disk degeneration of the spine in 257 siblings aged 43-85 years from probands of the Rotterdam Study.

	Age category (in years)						Total
	< 58	58-61	61-64	64-67	67-70	≥ 70	
Men	n = 21	n = 17	n = 20	n = 21	n = 14	n = 31	n = 124
Number with knee ROA (%)	2 (9.5)	3 (17.6)	2 (10.0)	4 (19.0)	1 (7.1)	4 (12.9)	16 (12.9)
Number with hip ROA (%)	2 (9.5)	1 (5.9)	0	0	1 (7.1)	2 (6.5)	6 (4.8)
Number with hand ROA (%)	9 (42.9)	13 (76.5)	8 (40.0)	16 (76.2)	11 (78.6)	25 (80.6)	82 (66.1)
Number with DD (%)	18 (85.7)	16 (94.1)	16 (80.0)	20 (95.2)	12 (85.7)	28 (90.3)	110 (88.7)
Women	n = 25	n = 12	n = 11	n = 21	n = 17	n = 47	n = 133
Number with knee ROA (%)	2 (8.0)	3 (25.0)	2 (18.2)	3 (14.3)	7 (41.2)	14 (29.8)	31 (23.3)
Number with hip ROA (%)	0	2 (16.7)	1 (9.1)	1 (4.8)	2 (11.8)	5 (10.6)	11 (8.3)
Number with hand ROA (%)	16 (64.0)	9 (75.0)	8 (72.7)	17 (81.0)	15 (88.2)	45 (95.7)	110 (82.7)
Number with DD (%)	16 (64.0)	8 (66.7)	7 (63.6)	15 (71.4)	16 (94.1)	41 (87.2)	103 (77.4)

DD = Disk degeneration of the spine. Numbers listed are numbers of individuals affected, with in brackets the percentage within the according age category. Definite ROA or disk degeneration was defined as Kellgren-score two or over (in case of the peripheral joints in the left and/or right corresponding joint).

motor disability, Heberden's nodes, family history of rheumatic diseases, occupation and work load, injuries and trauma's and comorbidity. As described earlier in Chapter 2.2 for the random sample from the Rotterdam Study, radiographs of the knees, hips, hands and spine were taken using identical standardized conditions. Two independent readers used the Kellgren grading system to score all radiographs (6), based on the protocol described in Chapter 2.2. Baseline characteristics of the 257 siblings that participated in the sibling pair study are shown in Table 1.

Prevalence of ROA and disk degeneration

The prevalence of ROA in the knees, hips, and hands and of disk degeneration of the spine in 124 men and 133 women aged 43 to 85 years is shown in Table 2. Hand ROA was defined as Kellgren-score two or over in any of the hand joints that was scored, i.e. 18 for each hand. Definite disk degeneration was defined as Kellgren-score two or over in any of the three levels that was scored.

In siblings of probands with ROA and disk degeneration at multiple joint sites ROA in peripheral joints is more frequent in female siblings as compared to male siblings. This was in keeping with the findings from the Rotterdam Study (see Tables 4 and 5, Chapter 2.2). In siblings, the frequency of disk degeneration

Table 3
Number of joint sites (knee, hip, hand, and spine) affected in 124 men and 133 women aged 43 to 85 years, siblings of probands with ROA at multiple sites.

Age category	Number of joint sites affected					Total
	0	1	2	3	4	
Men						
<58	2 (9.5)	10 (47.6)	6 (28.6)	3 (14.3)	0	21
58-61	1 (5.9)	2 (11.8)	11 (64.7)	3 (17.6)	0	17
61-64	2 (10.0)	12 (60.0)	4 (20.0)	2 (10.0)	0	20
64-67	1 (4.8)	4 (19.0)	12 (57.1)	4 (19.0)	0	21
≥67	1 (2.2)	12 (26.7)	25 (55.6)	6 (13.3)	1 (2.2)	45
Women						
<58	4 (16.0)	8 (32.0)	13 (52.0)	0	0	25
58-61	0	5 (41.7)	5 (41.7)	1 (8.3)	1 (8.3)	12
61-64	0	6 (54.5)	3 (27.3)	2 (18.2)	0	11
64-67	1 (4.8)	7 (33.3)	10 (47.6)	3 (14.3)	0	21
67-70	0	7 (10.9)	37 (57.8)	16 (25.0)	4 (6.3)	64

Numbers listed are numbers of individuals affected, with in brackets the percentage within the according age category. Definite ROA or disk degeneration was defined as a Kellgren-score two or over in the left and/or right corresponding joint.

of the spine was higher in men as compared to women, which was overall (88.7 percent in men versus 77.4 percent in women, see Table 2) statistically significant ($p = 0.02$). Within the hand, the DIP-joints were most often affected with ROA. The MCP-, CMC-1- and IP-joints were in both men and women about equally often affected with ROA, while in comparison herewith the frequency of PIP-joint ROA was slightly lower. Relatively rare were ROA in the carpal trapezoscaphoideal joint (TS) and the wrist joints (RN- and RU-joints).

In Table 3 the number of joint sites that is affected with ROA or disk degeneration is shown for men and women separately. Four different joint sites are considered, i.e. the knee, hip, hand, and spine. Out of the total number of siblings 15.3 percent of men and 20.3 percent of women have three or more joint sites affected with ROA and/or disk degeneration. However, 37.9 percent of all male siblings and 28.6 percent of all female siblings have zero or one joint site affected with ROA or disk degeneration. The latter percentages are substantially lower as compared to the frequencies observed in the Rotterdam Study (respectively 52.9 percent in men and 41.9 percent in women). In Chapter 3.2 we will study the familial clustering and heritability of ROA and disk degeneration based on these data.

References

1. Charcot JM. Clinical lectures on senile and chronic diseases. London, Payne T, 1803:148.
2. Nichols EH, Richardson FL. Arthritis deformans. J Med Res 1909;16:149-221.
3. Fassbender HG. Pathology of rheumatic diseases. New York, Springer-Verlag, 1975.
4. Begg MW, Scott JE. Hyaluronic acid and protein in simple ganglia and Heberden's nodes. Ann Rheum Dis 1966;25:145-8.
5. Burger H, van Daele PLA, Algra D, van den Ouweland FA, Grobbee DE, Hofman A, van Kuijk C, Schütte HE, Birkenhäger JC, Pols HAP. The association between age and bone mineral density in men and women aged 55 years and over. The Rotterdam Study. Bone Miner 1994;25:1-13.
6. Kellgren JH, Jeffrey MR, Ball J (eds). The epidemiology of chronic rheumatism. Volume II: Atlas of standard radiographs of arthritis. Oxford: Blackwell Scientific Publications, 1963.

3

Osteoarthritis in the General Population

Pattern of joint involvement and determinants of osteoarthritis at multiple sites in a population-based study

Abstract

Objective *To investigate the determinants for radiological osteoarthritis (ROA) and disk degeneration at multiple sites and to examine the clustering of ROA in the knees, hips, and hands and disk degeneration of the spine in the general population.*

Methods *A random sample of 1583 individuals, aged 55 to 70 years, was drawn from the Rotterdam Study. Radiographs of the knees, hips, hands, and thoracolumbar spine were scored for ROA and disk degeneration by means of the Kellgren grading system. Heberden's nodes were assessed in both hands. Multiple logistic regression analysis was used to estimate the odds ratio (OR) for the association of ROA and disk degeneration at multiple sites with the most important risk factors for OA. In separate multiple logistic regression analyses the clustering of joint involvement at the four different joint sites was examined with adjustments for the effects of age, body mass index (BMI), and bone mineral density (BMD) and stratified according to sex.*

Results *In women, BMI, BMD, and Heberden's nodes were besides age all statistically significant risk factors of polyarticular disease. The presence of knee ROA was significantly associated with radiological abnor-*

malities at the hip, hand and spine. In men, only BMI was in addition to age significantly associated with three or more affected joint sites and only polyarticular hand ROA was associated with disk degeneration of the spine.

Conclusion *Our results strongly support the existence of a subset of polyarticular OA in women. This is in agreement with a genetically determined susceptibility for cartilage degradation, which is modified by systemic factors. In men, our results were less equivocal.*

Introduction

Osteoarthritis (OA) is the most prevalent rheumatic disease. OA is not only a major cause of disability in the elderly but also the principal cause of knee and hip replacements (1,2). It is characterized by a progressive degeneration of hyaline cartilage and accompanying subchondral bone reaction of diarthrodial joints. The exact pathogenesis of OA is unknown, but exogenous as well as endogenous factors play a role in its etiology. Although some factors, such as age and sex, are determinants of OA at all joint sites, the role of most other factors has been reported to depend on the joint site of interest (3,4). This implies that OA is a clinically heterogeneous disorder that can be subdivided into discrete subsets, primarily based on descriptive definitions.

Kellgren and Moore suggested that the occurrence of OA in multiple joints, designated primary generalized OA, could be identified as a specific subtype of OA (5). They found evidence of a distinct pattern of joint involvement at multiple sites that was associated with Heberden's nodes. Other studies have focused on the effect of a distinct risk factor or of OA at a particular joint site on the occurrence of generalized OA (6-8). Generalized OA has a multifactorial etiology, involving hormonal, metabolic, mechanical and genetic influences. Women are more likely to have OA at multiple joint sites than men and in women there is evidence for a polyarticular subset of hand OA (7,9). Heberden's nodes may be predictors of OA in multiple joints, although their pathogenesis is not well understood. It has been suggested that these nodal deformities represent an inflammatory component in the etiology of generalized OA that, given their predominant presence in women, may be controlled by hormonal factors (10).

The classification criteria for generalized OA are a matter of debate and at the population level, the pattern of joint involvement in generalized OA has not been quantified in terms of prevalence of affected joint sites. Also, the role of risk factors that play a role in the occurrence of OA at individual joint sites is unclear in the etiology of generalized OA. The present study examines whether

the most important risk factors for OA are also determinants for radiological OA (ROA) and disk degeneration at multiple sites. Furthermore, we investigated the clustering of ROA in the knees, hips and hands and disk degeneration of the spine in a population based study.

Materials and Methods

Study population

The study was part of the Rotterdam Study; a prospective population based cohort study of occurrence and determinants of disease and disability in the elderly. Objectives and methods of the Rotterdam Study have been described in detail elsewhere (11). Briefly, all inhabitants of Ommoord, a district of the city of Rotterdam, aged 55 years or over were invited to participate. In total 7983 participants (response rate of 78 percent) were interviewed at home and examined extensively at the research center between 1990 and 1993. The medical ethics committee of Erasmus University Medical School granted permission to this study. Written informed consent was obtained from all participants.

A random sample of 1583 individuals, aged 55 to 70 years, was drawn from the total cohort of the Rotterdam Study. Radiographs of the peripheral joints, i.e. the knees, hips and hands, and of the thoracolumbar spine were scored for respectively ROA and disk degeneration in all individuals. Heberden's nodes were assessed in both hands separately, classified as absent or present, by trained investigators at the research center. Bone mineral density measurements of the femoral neck were performed using dual energy X-ray absorptiometry (Lunar DPX-L densitometer) as described previously (12). Height and weight were measured, with the participants in standing position without shoes. At the baseline interview, data was collected on joint complaints and morning stiffness.

Radiological OA and disk degeneration

Weight-bearing anterior-posterior radiographs of the pelvis and knees were obtained with respectively both feet in 10° endorotation and the patellae in central position. Furthermore, posteroanterior radiographs of the hands and wrists and lateral radiographs of the thoracolumbar spine (Th4-S1) were obtained.

Radiological determined criteria were used in accordance with other epidemiological studies concerning OA (3,9). We applied the Kellgren grading system (13), which incorporates the classic features of radiological OA, osteophyte formation and joint space narrowing. These radiological abnormalities reflect the pathophysiological changes in OA. The five point Kellgren grades (0-4), according to the figures and legends of the original atlas, were grade 0 =

normal; grade 1 = doubtful; grade 2 = minimal; grade 3 = moderate; grade 4 = severe. In the analysis definite ROA in a joint was defined as Kellgren-score two or over. Two trained observers, who had no knowledge of the other data of the participant, independently assessed all radiographs. After each set of about 150 radiographs the scores of the two observers were evaluated. Whenever the scores differed two or more points, or, was two for one observer but one for the other, a consensus score was agreed upon. Radiographs of the knees and hips had previously been scored in a similar fashion (14). ROA of the knee was assessed in the tibiofemoral joint. ROA of the hand was assessed in each inter- and metacarpalphalangeal, the first carpometacarpal, the trapezonavicular, the radionavicular and distal radioulnar joints.

ROA of the spine is confined to the apophyseal joints, but these joints could not be assessed at the lateral radiographs that were available. Although disk degeneration is not considered to be ROA of the spine, these radiological changes may be associated with ROA. Disk degeneration was scored using the Kellgren grades (0-4), in which a grade 0 or 1 denotes no or doubtful disk degeneration, a grade 2 denotes vertebral osteophytosis only and grades 3 and 4 vertebral osteophytosis accompanied by moderate or severe disk space narrowing. Three separate levels were scored, i.e. thoracic, lumbar and lumbosacral. Definite disk degeneration was defined as a Kellgren-score two or over in at least one level.

Statistical analysis

The prevalence of ROA at individual joint sites was calculated in three 5-year age strata for men and women separately. For examining ROA and disk degeneration at multiple sites we used data from four different joint sites, i.e. the knee, hip, hand and spine. In our study on familial aggregation of ROA and disk degeneration, we found hand ROA and disk degeneration of the spine to cluster in families (see Chapter 3.2). This suggests a common genetic origin in which cartilage degeneration is the unifying pathophysiologic hallmark. Knee ROA, hip ROA and disk degeneration of the spine were considered as dichotomous variables according to the absence or presence of this condition. To study polyarticular hand ROA, we categorized hand ROA according to the number of sites affected within the hands. Six separate sites within the hands were considered, i.e. the distal and proximal interphalangeal joints, the interphalangeal joint of the thumb, the metacarpal joints, the first carpometacarpal joint and the wrist joints.

Firstly, multiple logistic regression analysis was used to estimate the odds ratio (OR) for the association of ROA and disk degeneration at multiple sites with the most important risk factors for OA. The reference group in these analyses was the individuals free from ROA and disk degeneration. In gender specific analyses we compared individuals with one, two or three or more joint sites af-

fected with this reference group. The independent variables were age (continuous), bone mineral density of the femoral neck (continuous), body mass index (continuous), Heberden's nodes (dichotomous), morning stiffness (dichotomous) and joint complaints. Joint complaints were recorded in the knees, hips, hands, and lower back as absent or present. In the analyses joint complaints were categorized in three categories: no joint complaints (reference group), complaints in one joint site and complaints in two or more joint sites. Joint complaints should not be regarded as a determinant of ROA and disk degeneration, but rather as an indicator of the presence of clinical signs once ROA or disk degeneration has occurred.

Secondly, we investigated in separate multiple logistic regression analyses the clustering of joint involvement at the four different joint sites. The strength of the association between different joint sites was for each pair of joint sites summarized by an OR, which denotes the relative odds of having one site affected if the other site is also affected. To render these associations between pairs of joint sites independent of the effects of both other joint sites, all four joint sites were considered simultaneously in the model. All ORs were adjusted for age, bone mineral density, and body mass index and were stratified according to gender.

Logistic regression analyses were performed using SPSS statistical software. All ORs are presented with 95 percent confidence intervals (CI).

Results

Table 1 shows the prevalence of grade 2+ ROA at the knee, hip and hand joints and the prevalence of grade 2+ disk degeneration of the spine among 666 men and 917 women, stratified in 5 year strata. At all sites in both men and women the prevalence of ROA and disk degeneration increased with age. This increase was roughly equal in men and women, except for hip ROA, in which joint in women the prevalence increased fourfold between ages 55 and 70 years. The prevalence of knee ROA and hand ROA was at any age between 55 and 70 years higher in women than in men. Hip ROA was below the age of 65 years more prevalent in men, but after this age the prevalence of hip ROA rose steeply in women. Disk degeneration of the spine was the most prevalent condition, occurring in similar frequencies in men and women.

Table 2 shows the association between the number of joint sites affected with ROA and disk degeneration and its most important determinants among women. In women, in case that one of four joint sites is affected, only age was statistically significantly associated with the presence of ROA and disk degeneration. Age, bone mineral density, and Heberden's nodes were statistically significant determinants of two affected joint sites. In case three or more joint sites

Table 1
Distribution of knee ROA, hip ROA, disk degeneration of the spine and hand ROA per age stratum of 5 years in 666 men and 917 women, aged 55-70 years, from the Rotterdam Study.

	Men				Women			
	55-60 years	60-65 years	65-70 years	Total	55-60 years	60-65 years	65-70 years	Total
	frequency (n)	frequency (n)	frequency (n)	n	frequency (n)	frequency (n)	frequency (n)	n
*Knee ROA**								
No	92.2 (141)	87.0 (208)	83.6 (224)	573	85.0 (210)	77.5 (265)	75.9 (242)	717
Yes	7.8 (12)	13.0 (31)	16.4 (44)	87	15.0 (37)	22.5 (77)	24.1 (77)	191
Hip ROA†								
No	89.5 (136)	88.2 (210)	87.1 (236)	582	95.5 (236)	93.3 (319)	81.8 (260)	815
Yes	10.5 (16)	11.8 (28)	12.9 (35)	79	4.5 (11)	6.7 (23)	18.2 (58)	92
DD Spine‡								
No	40.1 (61)	30.1 (71)	24.5 (66)	198	40.1 (99)	36.5 (123)	20.2 (65)	287
Yes	59.9 (91)	69.9 (165)	75.5 (203)	459	59.9 (148)	63.5 (214)	79.8 (256)	618
Hand ROA								
No	61.9 (96)	50.8 (122)	45.0 (122)	340	44.0 (110)	29.6 (102)	23.3 (75)	287
1 joint site	27.7 (43)	25.4 (61)	26.6 (72)	176	35.2 (88)	31.3 (108)	22.4 (72)	268
2 joint sites	8.4 (13)	12.1 (29)	15.5 (42)	84	11.6 (29)	18.8 (65)	23.6 (76)	170
≥ 3 joint sites	1.9 (3)	11.7 (28)	12.9 (35)	66	9.2 (23)	20.3 (70)	30.7 (99)	192

* Knee radiographs were missing for 15 subjects.
† Hip radiographs were missing for 15 subjects.
‡ Radiographs of the spine were missing for 21 subjects.

are affected also body mass index became a statistically significant determinant. The prevalence of polyarticular disease in women, defined as three or more joint sites affected, increased with every year increase in age by 24 %, with every 0.01 g/cm^2 increase in bone mineral density this prevalence increased with 8 %. Every 1.0 kg/m^2 increase in body mass index increased the prevalence of polyarticular disease with 10 %. When Heberden's nodes are present this prevalence increased threefold (OR = 3.01, 95 % CI 1.29-7.00). Joint complaints at two or more joint sites were 2.59 times (95% CI 1.03-6.53) more likely to be present in women with three or more joint sites affected as compared to women with no ROA and no disk degeneration. All risk factors that were statistically significantly associated with polyarticular disease showed an increasing OR with increasing number of joint sites affected.

Table 3 shows the association between the number of joint sites affected with ROA and disk degeneration and its most important determinants among men. In men, as was the case in women, only age was associated with one affected joint site. Body mass index was a statistically significant determinant for men with two or more joint sites affected with ROA and disk degeneration. For Heberden's nodes a significant association was only observed in men with two

Table 2
Determinants of ROA and/or disk degeneration according to the number of joint sites affected in 917 women (numbers shown are odds ratios with 95 % CI).

Determinant	Number of joint sites affected†		
	1 joint site (n = 274)	2 joint sites (n = 360)	≥ 3 joint sites (n = 158)
Age in years	1.08 (1.02-1.16)*	1.21 (1.13-1.30)*	1.24 (1.13-1.36)*
BMD in cg/cm^2 ‡	1.01 (0.99-1.04)	1.03 (1.01-1.06)*	1.08 (1.04-1.11)*
BMI in kg/m^2	1.03 (0.96-1.10)	1.03 (0.95-1.11)	1.10 (1.01-1.21)*
Heberden's nodes (present vs absent)	1.36 (0.69-2.71)	2.30 (1.17-4.52)*	3.01 (1.29-7.00)*
Morning stiffness (present vs absent)	1.10 (0.61-1.98)	1.33 (0.74-2.39)	1.05 (0.50-2.19)
Complaints at:			
1 joint site	0.78 (0.43-1.44)	1.14 (0.61-2.13)	2.40 (1.00-5.78)
≥ 2 joint sites	0.95 (0.47-1.92)	0.83 (0.40-1.71)	2.59 (1.03-6.53)*

* $p < 0.05$.
† Four different joint sites were considered, i.e. the knee, hip, hand and spine. The reference group in these analyses was the individuals free from ROA and disk degeneration at all four joint sites (n = 100).
‡ Bone mineral density was expressed in cg/cm^2 . BMD = bone mineral density. BMI = body mass index.

joint sites affected. Neither bone mineral density nor morning stiffness was significantly associated with the presence of ROA and disk degeneration in men. Joint complaints at two or more joint sites were 3.92 times (95% CI 1.17-13.2) more likely to be present in men with polyarticular disease as compared to men with no joint sites affected. In men, the ORs for all factors, except Heberden's nodes and morning stiffness, increased with increasing number of joint sites affected.

The associations between the four individual joint sites, showing the pattern of clustering for different joint sites, are shown in Tables 4 and 5 for respectively women and men. In women, the presence of knee ROA was statistically significantly associated with the presence of hip ROA, hand ROA and disk degeneration of the spine. The strongest association was found between knee ROA and hand ROA in three or more joint sites (OR = 2.94, 95 % CI 1.72-5.03); see Table 4. Disk degeneration of the spine in women was associated with hand ROA in three or more joint sites (OR = 1.76, 95 % CI 1.09-2.83). This latter association was also observed in men (OR = 2.63, 95 % CI 1.19-5.82); see Table 5. In men, there was no statistically significant evidence for clustering of ROA and/or disk degeneration at other sites.

Table 3
Determinants of ROA and/or disk degeneration according to the number of joint sites affected in 666 men (numbers shown are odds ratios with 95 % CI).

Determinant	Number of joint sites affected†		
	1 joint site (n = 249)	2 joint sites (n = 236)	≥ 3 joint sites (n = 70)
Age in years	1.08 (1.02-1.16)*	1.16 (1.08-1.25)*	1.26 (1.13-1.40)*
BMD in cg/cm^2 ‡	1.00 (0.98-1.02)	1.01 (0.98-1.03)	1.02 (0.99-1.05)
BMI in kg/m^2	1.05 (0.96-1.16)	1.15 (1.03-1.27)*	1.20 (1.04-1.39)*
Heberden's nodes (present vs absent)	1.67 (0.64-4.31)	3.08 (1.22-7.82)*	2.97 (0.86-10.3)*
Morning stiffness (present vs absent)	0.95 (0.51-1.79)	0.87 (0.44-1.70)	2.05 (0.86-4.86)
Complaints at:			
1 joint site	1.15 (0.64-2.06)	1.53 (0.82-2.84)	1.92 (0.78-4.76)
≥ 2 joint sites	1.82 (0.75-4.44)	1.95 (0.78-4.86)	3.92 (1.17-13.2)*

* $p < 0.05$.
† Four different joint sites were considered, i.e. the knee, hip, hand and spine. The reference group in these analyses was the individuals free from ROA and disk degeneration at all four joint sites (n = 95).
‡ Bone mineral density was expressed in cg/cm^2 . BMD = bone mineral density. BMI = body mass index.

Table 4
Pattern of joint involvement in radiological osteoarthritis (ROA) of the knee, hip, and hand and disk degeneration of the spine in women.

	Knee ROA OR (95 % CI)	Hip ROA OR (95 % CI)	DD Spine OR (95 % CI)
Knee ROA	–		
Hip ROA	1.84 (1.07-3.18)*	–	
DD Spine	1.62 (1.05-2.50)*	1.58 (0.84-2.97)	–
Hand ROA:			
1 site	1.60 (0.95-2.68)	1.47 (0.73-2.96)	1.07 (0.74-1.55)
2 sites	1.84 (1.05-3.23)*	0.87 (0.38-1.97)	1.28 (0.82-2.00)
≥ 3 sites	2.94 (1.72-5.03)*	1.68 (0.82-3.40)	1.76 (1.09-2.83)*

* $p < 0.05$.
All ORs are adjusted for age, body mass index and bone mineral density.
DD Spine = Disk degeneration of the spine.

Table 5
Pattern of joint involvement in radiological osteoarthritis (ROA) of the knee, hip, and hand and disk degeneration of the spine in men.

	Knee ROA OR (95 % CI)	Hip ROA OR (95 % CI)	DD Spine OR (95 % CI)
Knee ROA	–		
Hip ROA	1.62 (0.83-3.18)	–	
DD Spine	0.98 (0.56-1.72)	1.24 (0.69-2.23)	–
Hand ROA:			
1 site	0.78 (0.42-1.45)	1.58 (0.87-2.88)	1.03 (0.68-1.56)
2 sites	1.11 (0.53-2.30)	1.84 (0.88-3.85)	1.34 (0.75-2.40)
≥ 3 sites	1.19 (0.55-2.57)	1.40 (0.58-3.34)	2.63 (1.19-5.82)*

* $p < 0.05$.
All ORs are adjusted for age, body mass index and bone mineral density.
DD Spine = Disk degeneration of the spine.

Discussion

Our results strongly support the existence of a subset of polyarticular OA in women. In women, body mass index, bone mineral density, and Heberden's nodes were besides age all statistically significant risk factors of ROA and disk degeneration in three or more joint sites. In women, the presence of knee ROA was statistically significantly associated with radiological abnormalities at the hip, hand and spine. This latter finding was independent of the effects of age,

body mass index and bone mineral density. In men our results were less equivocal. In men, only body mass index was in addition to age statistically significantly associated with three or more affected joint sites. Moreover, only polyarticular hand ROA was associated with disk degeneration of the spine.

A limitation of our study is the lack of data on the patellofemoral joint, the cervical spine, the apophyseal joints of the spine, and joints of the feet. This resulted most likely in an underestimation of the clustering of ROA and/or disk degeneration and may have diminished the detected associations. As all radiographs were scored irrespective of the other data of the participant studied, misclassification will most likely have been randomly distributed across the participants, making the occurrence of spurious associations due to these omissions unlikely.

Several studies have suggested that in the occurrence of OA at different joint sites different risk factors play a role (15,16). In the present study, age was the only risk factor associated with the presence of ROA or disk degeneration at one joint site. In women, the most important known risk factors for OA, i.e. age, body mass index and bone mineral density were all statistically significantly associated with polyarticular disease. In men, polyarticular disease was associated with age and body mass index. These findings suggest a generalized effect, i.e. not acting joint site specific, of body mass index on the occurrence of ROA and disk degeneration. In women, bone mineral density was found to have a similar generalized effect. Our findings are compatible with the hypothesis of a susceptibility of genetic origin, which gives rise to OA whenever additionally one or more systemic risk factors are present. The clustering of knee ROA with ROA at the hip and hand and with disk degeneration of the spine, adjusted for age, body mass index, and bone mineral density, supports this hypothesis of a generalized susceptibility in polyarticular OA. The association of disk degeneration of the spine with knee ROA in women and with polyarticular hand ROA in both men and women suggests that peripheral ROA and disk degeneration share a common pathogenesis. As these associations were independent of the influence of systemic risk factors for OA, this common pathogenesis may be genetically determined. To our knowledge this is the first study suggesting that disk degeneration of the spine can be included in the concept of polyarticular OA in women.

The role or meaning of Heberden's nodes in generalized OA has been unclear. The present study shows that in both men and women Heberden's nodes are strong predictors of polyarticular disease, although in men with three or more joint sites affected this was not statistically significant. In an analysis in which we stratified according to the presence or absence of Heberden's nodes, the associations with age, body mass index, and bone mineral density were not essentially different in individuals with Heberden's nodes as compared to indi-

viduals without Heberden's nodes (data not shown). However, in those with Heberden's nodes we found a strong association between polyarticular disease (three or more joint sites affected) and morning stiffness in both men and women. This finding suggests that the presence of Heberden's nodes is associated with an inflammatory component in the pathogenesis of OA, giving rise to a more aggressive progression of the disease and/or complaints. This is in line with the high prevalence of Heberden's nodes that is usually observed in clinical OA.

Two earlier population-based studies have examined the pattern of joint involvement in generalized OA (7,17). A British study by Cooper et al. suggested the existence of polyarticular OA in women, based on the association of knee ROA with hip and hand ROA (17). Neither men nor the role of putative risk factors were examined. An American study by Hirsh et al. showed evidence for the existence of polyarticular disease in both men and women based on the association of knee and hand ROA (7). For women the findings of the present study were consistent with both previous studies. For men our findings were less outspoken, as clustering of ROA was restricted to polyarticular hand ROA and disk degeneration of the spine.

In conclusion, our results strongly support the existence of a subset of polyarticular OA in women. In women, age, body mass index, and bone mineral density were all statistically significantly associated with polyarticular disease. In men, this was only observed for age and body mass index. For women, our findings are in keeping with a genetically determined susceptibility for cartilage degradation, which is modified by systemic factors.

References

1. Felson D'T. Epidemiology of hip and knee osteoarthritis. Epidemiol Rev 1988;10:1-28.
2. Bulstrode C. Keeping up with orthopaedic epidemics. BMJ 1987;295:514.
3. Cerhan JR, Wallace RB, El-Khoury GY, Moore TE. Risk factors for progression to new sites of radiographically defined osteoarthritis in women. J Rheumatol 1996;23:1565-78.
4. Peyron JG. The epidemiology of osteoarthritis. In: Moskowitz RW, Howell DS, Goldberg VM, Mankin HS, eds. Osteoarthritis: diagnosis and management. Saunders: Philadelphia and London, 1984:9-27.
5. Kellgren JH, Moore R. Generalised osteoarthritis and Heberden's nodes. BMJ 1952;1:181-7.
6. Kellgren JH, Lawrence JS, Bier F. Genetic factors in generalized osteo-arthrosis. Ann Rheum Dis 1963;22:237-54.
7. Hirsh R, Lethbridge-Cejku M, Scott Jr WW, Reichle R, Plato CC, Tobin J, Hochberg MC. Association of hand and knee osteoarthritis: evidence for a polyarticular disease subset. Ann Rheum Dis 1996;55:25-9.

8. Croft P, Cooper C, Wickham C, Coggon D. Is the hip involved in generalized osteoarthritis? Br J Rheumatol 1992;31:325-8.
9. Egger P, Cooper C, Hart DJ, Doyle DV, Coggon D, Spector TD. Patterns of joint involvement in osteoarthritis of the hand: the Chingford Study. J Rheumatol 1995;22:1509-13.
10. Spector TD, Campion GD. Generalized osteoarthritis: a hormonally mediated disease. Ann Rheum Dis 1989;48:523-7.
11. Hofman A, Grobbee DE, De Jong PTVM, Van de Ouweland FA. Determinants of disease and disability in the elderly. Eur J Epidemiol 1991;7:403-22.
12. Burger H, van Daele PLA, Algra D, van den Ouweland FA, Grobbee DE, Hofman A, van Kuijk C, Schütte HE, Birkenhäger JC, Pols HAP. The association between age and bone mineral density in men and women aged 55 years and over: the Rotterdam Study. Bone Miner 1994;25:1-13.
13. Kellgren JH, Jeffrey MR, Ball J (eds). The epidemiology of chronic rheumatism. Volume II: Atlas of standard radiographs of arthritis. Oxford: Blackwell Scientific Publications, 1963.
14. Odding E, Valkenburg HA, Algra D, Van den Ouweland FA, Grobbee DE, Hofman A. Associations of radiological osteoarthritis of the hip and knee with locomotor disability in the Rotterdam Study. Ann Rheum Dis 1998;57:203-8.
15. Felson DT, Zhang Y. An update on the epidemiology of knee and hip osteoarthritis with a view to prevention. Arthritis Rheum 1998;41:1343-55.
16. Creamer P, Hochberg MC. Osteoarthritis. Lancet 1997;350:503-8.
17. Cooper C, Egger P, Coggon D, Hart DJ, Masud T, Cicuttini F, Doyle DV, Spector TD. Generalized osteoarthritis in women: pattern of joint involvement and approaches to definition for epidemiological studies. J Rheumatol 1996;23:1938-42.

Heritabilities of radiological osteoarthritis in peripheral joints and of disk degeneration of the spine

Abstract

Objective *To estimate the genetic influence on the occurrence of radiological osteoarthritis (ROA) in the knees, hips and hands and disk degeneration of the spine in the general population.*

Methods *A random sample of 1583 individuals was drawn to estimate the prevalence of ROA and disk degeneration in the general population. Of 118 probands with multiple affected joint sites, derived from this sample, we were able to recruit 257 siblings. The variance of ROA and disk degeneration within sibling pairs was compared with the variance between pairs of siblings. Heritability estimates for ROA in the knees, hips, and hands and for disk degeneration of the spine were calculated. Osteoarthritis (OA) was defined on radiological criteria, using Kellgren's grading system.*

Results *We observed that hand ROA and disk degeneration of the spine were statistically significantly more frequent in siblings as compared with the random sample, whereas the prevalence of knee and of hip ROA was similar and lower, respectively. Heritability estimates for hand ROA and disk degeneration were statistically significant, respectively 0.56 (95 percent CI 0.34-0.76) and 0.75 (95 percent CI 0.30-1.00). For knee and hip*

ROA no evidence for a genetic effect in the general population was found. Finally, the heritability estimate for a score summing the number of joints affected in the knees, hips, hands and spine was 0.78 (95 percent CI 0.52-0.98). All heritability estimates were adjusted for age, sex, body mass index, and bone mineral density.

Conclusion *The present study shows that in the general population a strong genetic effect exists for hand ROA and disk degeneration of the spine. The findings on the total number of joints affected at multiple sites suggest genetic susceptibility to generalized OA.*

Introduction

A genetic effect on osteoarthritis (OA) was initially recognized in 1941 by Stecher, who showed that Heberden's nodes of the fingers were more common in sisters of affected subjects than in the population (1). In 1963, Kellgren et al (2) reported, in a study of subjects derived from an out-patient clinic, that first degree relatives of subjects with generalized radiological OA (ROA) were twice as likely to be affected with ROA than expected in the population. In 1996, Spector et al (3) measured ROA in hands and knees in female twins and suggested that genetic factors might explain up to 65 percent of the variability in ROA of the hand and knee. This familial aggregation of hand and knee ROA was confirmed recently in two population-based studies (4,5). Furthermore, Felson et al (4) found evidence, in a segregation analysis, for effects of a major recessive gene with a residual multifactorial component.

With the exception of the study by Kellgren et al (2), previous studies concerning the role of genetic factors in the occurrence of ROA were limited to OA of the hands and knees (3-5). The contribution of genetic factors to other common forms of OA, including hip ROA and disk degeneration of the spine, has not been studied. Two studies addressed the role of body mass index in the familial aggregation of ROA (4,5), but none of the previous studies examined the role of bone mineral density. This is important because bone mineral density, like body mass index, is a risk factor for OA and is strongly influenced by genetic factors (6,7).

We performed a population-based study on the contribution of genetic factors to the occurrence of ROA in the knees, hips, and hands and of disk degeneration in the spine. Heritability estimates for ROA and disk degeneration were calculated, using data of a random sample of 1583 individuals and 257 siblings of subjects with ROA and disk degeneration at multiple joint sites.

Methods

Subjects

The study was embedded in the Rotterdam Study; a prospective population based follow-up study of determinants and prognosis of chronic diseases in the elderly (8). All persons living in Ommoord, a suburb of Rotterdam, aged 55 years and over were invited to participate. In total 7983 participants (response rate of 78 percent) were examined. The medical ethics committee of Erasmus University Medical School has approved the study and written informed consent was obtained from all participants.

`In order to quantify the occurrence of ROA and disk degeneration in the general population, we have drawn a random sample of 1583 individuals aged 55 to 70 years from the Rotterdam Study. Radiographs of the peripheral joints, i.e. the knees, hips and hands, and of the thoracolumbar spine were scored for respectively ROA and disk degeneration in all individuals. To estimate the genetic component in the etiology of ROA and disk degeneration, we studied the siblings of a subsample of 250 probands (aged between 55 and 65 years) with ROA and disk degeneration in at least two joint sites. In this relatively young age category, genetic predisposition may play a more predominant role than in the elderly, in whom environmental factors and aging may be more important determinants. Probands were selected based on the radiological abnormalities present on the radiographs of the knees, hips, hands, and spine. Probands had to have two or more of these four joint sites affected. Except when individuals had hand ROA in combination with disk degeneration of the spine, both highly prevalent conditions, which are likely to occur in combination by chance in high frequency. In that case, a proband had to have additional Heberden's nodes in order to reduce the number of siblings to be studied. This selection of probands was carried out in order to ascertain the group with the highest a priori probability of genetic factors playing a role in the occurrence of ROA.

Of the 221 probands that were willing to participate (response rate of 88 percent), 24 had no siblings. The remaining 197 probands had a total of 708 siblings born alive. Of these 708 siblings, 168 were deceased, 63 lived abroad, 65 could not be contacted, 24 were not able to participate because of a disease other than OA, and for 20 individuals the reason for not participating was unknown. From the 368 siblings that could be contacted, we were able to recruit 257 siblings (70 percent). These 257 siblings were derived from 118 probands and were examined at the research center. A majority (64 percent) of the 111 siblings that upon request refused to enter into the study indicated that they had no particular reason to do so.

Measurements

For all individuals the following radiographs were obtained: weight bearing anterior-posterior pelvic radiographs with both feet in 10°endorotation, weight bearing knee radiographs with the patellae in central position, anteroposterior radiographs of the hands and wrists and lateral radiographs of the spine (Th4-S1).

The exact definition of OA remains a matter of debate, but the use of radiologically determined changes as can be seen on radiographs is widely accepted in epidemiological research concerning OA (9,10). We used the Kellgren grading system (11) for all joint sites, since it incorporates both classic features of radiological OA, osteophyte formation and joint space narrowing and to our opinion up until now no convincing evidence has been brought forward to regard these features as completely independent markers of disease. Thus, ROA was assessed by means of the Kellgren grading system in five grades (0-4), using the figures and legends of the original atlas. Two independent readers, who had no knowledge of the other data of the participant, scored all radiographs. After each set of about 150 radiographs the scores of the two readers were evaluated, in order to reduce bias related to intra- and inter-rater agreement. Whenever the scores were two or more points different, or, was two for one reader but one for the other, a consensus score was agreed upon. Previous validation studies concerning the use of the Kellgren grading system showed that full agreement between observers varied from 82 percent in case of hip ROA to 60 percent in case of disk degeneration of the spine (12). ROA of the knee was only assessed in the tibiofemoral joint. ROA of the hand was assessed in each inter- and metacarpal-phalangeal joint, the first carpometacarpal, the trapezoscaphoideal, the radionavicular and distal radioulnar joints.

By definition, ROA of the spine is confined to the apophyseal joints, but these joints could not be assessed on the lateral radiographs of the spine that were available. In stead, we assessed disk degeneration of the spine, of which the genetic etiology may be associated with the occurrence of ROA in the peripheral joints (13). Disk degeneration was scored using the Kellgren grades (0-4), in which a grade zero or one denotes no or doubtful disk degeneration, a grade two denotes vertebral osteophytosis only and grades three and four vertebral osteophytosis accompanied by moderate or severe disk space narrowing. Three levels were scored separately, i.e. thoracic, lumbar and lumbosacral.

The presence of Heberden's nodes was determined by an examination of the hands, which was performed by trained investigators at the research center. Heberden's nodes were scored in both hands separately, classified as absent or present, without knowledge of the radiographic findings. Bone mineral density was measured at the femoral neck by dual energy x-ray absorptiometry as de-

scribed previously (14). Weight and height were measured at the research center according to standardized procedures.

Classification of ROA

A total of 36 separate joints in the hands were scored for ROA. These joints in the hands were grouped into 8 different groups of joints: distal interphalangeal (DIP), interphalangeal joint of the thumb (IP), proximal interphalangeal (PIP), metacarpalphalangeal (MCP), first carpometacarpal (CMC I), trapezoscaphoideal (TS), radionavicular (RN) and distal radioulnar (RU). This rendered 16 groups of joints (right and left separate). Together with the right and left knee and hip joints and the three levels in the spine, the total number of joint groups consisted of 23. We constructed a sum score to be able to summarize the presence of ROA in the peripheral joints and disk degeneration in the spine as a quantitative trait. Each one of the 23 joint groups that had been scored, contributed one point to the sum score in the case the Kellgren-score in this joint group was two or over.

Furthermore, in the analysis each joint site, i.e. the knee, hip, hand and spine, was examined separately. In these analyses, the hands were regarded as one joint group, consisting of 36 individual joints and 16 groups of joints in both hands together. Hand ROA was analyzed as a semi-continuous trait, with a trait score equaling the number of joint groups in the hands with a Kellgren-score two or over. Knee and hip ROA were analyzed as dichotomous traits, with definite ROA defined as Kellgren score two or over in the right or left corresponding joint. The spine was also regarded as one joint group, consisting of three different levels, i.e. thoracic, lumbar and lumbosacral. Definite disk degeneration was defined as Kellgren score two or over in any of the three levels scored.

Statistical analysis

Demographic variables in the population and siblings were compared using Student's *t* test and chi-square test. Distributions of the sum score of ROA and disk degeneration were non-parametrically tested using Mann-Whitney test. Heritability is defined as the ratio of all genetic variance to the total variance and was estimated in two steps. Firstly, the variance of ROA and disk degeneration within a sibling pair was compared with the variance between pairs of siblings. If a genetic effect is present, the variance within a sibling pair is expected to be lower than the variance between pairs of siblings. Secondly, we used the data on the variances of ROA and disk degeneration in the random sample of 1583 individuals, to derive heritability estimates for the general population. Correspondingly, heritability estimates were calculated for body mass index and bone mineral density of the femoral neck. Although we can not exclude a role of

shared environmental factors early in life, the influence of shared environment on the occurrence of ROA at late middle age is expected to be limited, given that siblings lead separate lives. Furthermore, the correlation of OA in spouses has been found to be low (4), suggesting the absence of a strong environmental factor. At the individual level adjustments were made for known genetic risk factors for ROA, which siblings may share, including age, sex, body mass index and bone mineral density.

To calculate the heritability estimates, a random effects model, in which random effects represent genetic effects (15), was fitted using maximum likelihood estimation (16). For the normally distributed outcomes, i.e. sum score of ROA and disk degeneration (log transformed), hand ROA (log transformed), body mass index and bone mineral density of the femoral neck, a linear model was used. A logistic model was used for the binary outcomes, i.e. knee ROA, hip ROA and disk degeneration of the spine. Heritability estimates are presented with 95 percent confidence intervals (CI). The heritability estimates for ROA and disk degeneration are applicable to the general population under the assumptions that, the sample drawn from the Rotterdam Study is a random sample from the population, the genetic variance within a sibling pair is independent of ROA status, and the influence of shared environment on this late onset disease is limited.

Results

Characteristics of the 118 probands, their 257 corresponding siblings, and the random population based sample of 1583 individuals, from which the probands were derived, are shown in Table 1. Four joint sites were affected in 5 probands, 47 probands had three affected joint sites, and 66 probands had a combination of two joint sites affected. Of the 76 probands with at least hand ROA and disk degeneration of the spine, the combination most frequently affected, 28 had additionally Heberden's nodes. Siblings were recruited about four years after the probands had been examined at the research center (see Table 1). The frequency of knee ROA in siblings was similar to the frequency found in the total sample. The frequency of hip ROA in siblings was lower than would be expected based on the data from the total sample. Hand ROA and disk degeneration was significantly more frequent in siblings as compared to the total sample. The distributions of the sum score of ROA and disk degeneration, expressing the total number of joints affected, for probands, siblings, and the random sample derived from the Rotterdam Study are shown in Figure 1. This figure shows that the distribution for siblings is positioned in between the distributions of the probands and the total sample. The median of the sum score in siblings was signifi-

Table 1
Characteristics of probands (n = 118) and their corresponding siblings (n = 257) in relation to a random population sample (n = 1583), from which the probands were derived.

	Male probands n = 33	Male siblings n = 124	Men total sample n = 666	Female probands n = 85	Female siblings n = 133	Women total sample n = 917
Age (SD) in years Range	61.1 (2.7) 56 – 65	64.7 (7.3)* 45 – 82	63.4 (4.1) 55-70	60.8 (2.7) 55 – 65	65.8 (8.5)* 43 – 85	62.9 (4.1) 55-70
Body mass index (SD) in kg/m²	27.2 (2.4)	26.3 (3.0)	25.9 (3.0)	27.6 (4.7)	27.1 (4.7)	26.6 (4.2)
Bone mineral density (SD) in g/cm²	0.93 (0.13)	0.88 (0.14)	0.89 (0.13)	0.90 (0.14)	0.84 (0.15)	0.83 (0.13)
Number with knee ROA (%)	14 (42.4)	16 (12.9)	87 (13.1)	56 (65.9)	31 (23.3)	191 (20.8)
Number with hip ROA (%)	12 (36.4)	6 (4.8)†	79 (11.9)	18 (21.2)	11 (8.3)	92 (10.0)
Number with hand ROA (%)	24 (72.7)	82 (66.1)*	326 (48.9)	73 (85.9)	110 (82.7)*	630 (68.7)
Number with disk degeneration (%)	28 (84.8)	110 (88.7)*	456 (68.5)	67 (78.8)	103 (77.4)†	607 (66.2)
Median of sum score ‡	3.0 (2,8)	3.0 (1,9)§	2.0 (0,6)	5.0 (2,9)	5.0 (1,10)§	3.0 (0,7)

* $p \leq 0.001$ and † $0.01 > p < 0.05$ (siblings compared with total cohort for men and women separately).
‡ Sum score of ROA and disk degeneration with in brackets the 10th and 90th percentile values.
§ $p < 0.001$ (siblings compared with total sample using Mann-Whitney test).
ROA = radiological osteoarthritis.

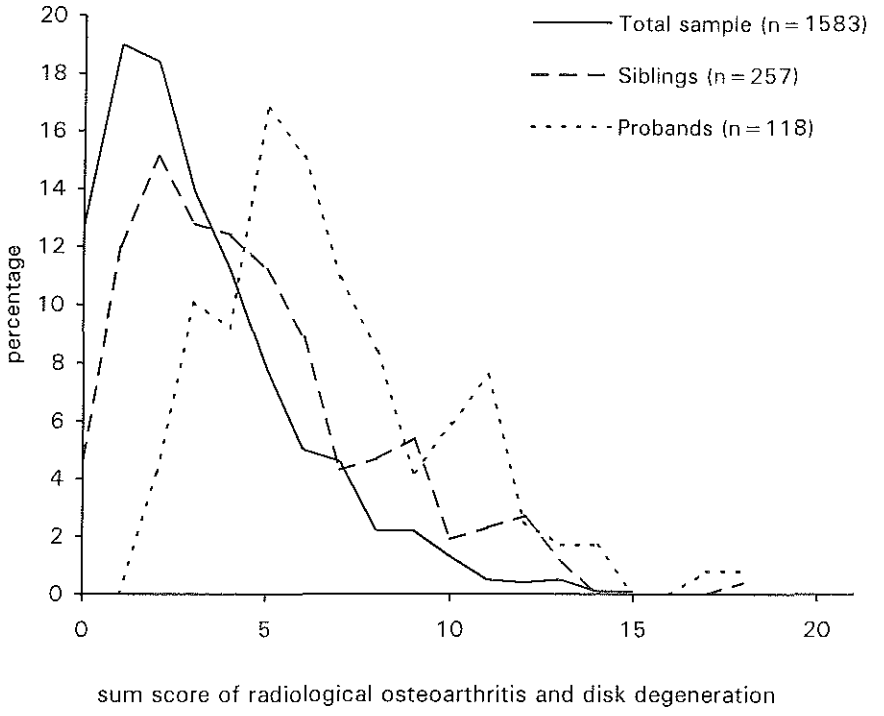


Figure 1
Distribution of the sum score of radiological osteoarthritis and disk degeneration of the spine in probands, siblings, and the total population-based sample.

Table 2
Heritability estimates for ROA, disk degeneration, body mass index and bone mineral density.

	Heritability estimate (95 percent CI)
Sum score of ROA and disk degeneration	0.78 (0.52 – 0.98)*
Knee ROA	0.07 (0.00 – 0.41)* (NS)
Hand ROA	0.56 (0.34 – 0.76)*
Disk degeneration of the spine	0.75 (0.30 – 1.00)*
Body mass index	0.53 (0.34 – 0.75)†
Bone mineral density of the femoral neck	0.52 (0.25 – 0.70)†

*Adjusted for age, sex, body mass index and bone mineral density.
† Adjusted for age and sex.
ROA = radiological osteoarthritis. NS = not significant. CI = confidence interval.
NB: For hip ROA no heritability estimate was calculated as the frequency of hip ROA in siblings was lower than that in the random population.

cantly higher than the median of the sum score found in the random sample and was similar to that in probands.

The heritability estimate of the sum score of ROA and disk degeneration was 0.78 (95 percent CI 0.52-0.98), after adjustment for age, sex, body mass index and bone mineral density (Table 2). This implies that up to 78 percent of the variance in the sum score of ROA and disk degeneration is explained by genetic factors, independent of the influences of age, sex, body mass index and bone mineral density. For the individual joint sites, heritability estimates are given in Table 2. In these analyses, hand ROA was analyzed as a normally distributed trait, according to the number of joint groups affected in both hands (at maximum 16). Knee and hip ROA, and disk degeneration of the spine were analyzed as dichotomous traits. Table 2 shows that disk degeneration had the highest heritability (0.75 with 95 percent CI 0.30-1.00). Hand ROA was also statistically significantly correlated in siblings (heritability estimate: 0.56 with 95 percent CI 0.34-0.76). However, ROA in the knee was not statistically significantly correlated in family members. The frequency of hip ROA in siblings was even lower than that in the random sample. Only higher than expected concordance rates in siblings can be explained biologically, therefore no heritability estimate was calculated for hip ROA.

Heritability estimates for body mass index and bone mineral density of the femoral neck were also statistically significant, independent of age and sex, 0.53 (95 percent CI 0.34-0.75) and 0.52 (95 percent CI 0.25-0.70), respectively.

Discussion

The present study suggests that genetic factors play a substantial role in the occurrence of radiological OA in the general population. Siblings of subjects with ROA and disk degeneration at multiple joint sites had higher frequencies of hand ROA and disk degeneration. However, the frequencies of knee ROA and hip ROA were equal and lower, respectively, as compared to a random sample of individuals derived from the Rotterdam Study. When considering the total number of joints affected at four separate sites, i.e. the knees, hips, hands and spine, we found that up to 78 percent of the total variance of this sum score of ROA and disk degeneration was explained by genetic factors. In particular hand ROA and disk degeneration of the spine showed a statistically significant aggregation in siblings. Interestingly, the genetic influence on ROA established here is independent of the well-known genetic influences present in body mass index and bone mineral density, which are also observed in this study. Since heritability estimates were calculated with the use of both the data from the sibling pairs and a random population based sample from which the probands were selected,

these estimates are applicable to the general population (16). Our data suggest the existence of a genetic susceptibility to generalized cartilage degeneration, independent of the genetic influences in body mass index and bone mineral density.

We compared the findings of the three previous studies on the heritability of OA, including a twin study and two population-based studies (3-5), with the results of the present study. For the sake of comparability we also calculated sib-sib correlations. Except for the twofold higher correlation of hand ROA between siblings in the study by Hirsch et al. (5), the sib-sib correlations in all four studies were reasonably similar, despite differences between studies in type of study population and definition criteria for ROA. The present study is the first to report an increased frequency of disk degeneration in siblings of probands with ROA and disk degeneration, suggesting a shared genetic etiology for ROA and disk degeneration in humans. Furthermore, the present study suggests a genetic predisposition for ROA and disk degeneration at multiple sites as measured by the sum score. Although two earlier studies have examined familial aggregation of ROA by summing the number of joints affected, this is the first study to include hip ROA and disk degeneration of the spine in the sum score (4,5). Disk degeneration in addition to hand ROA showed the highest heritability estimates, whereas the evidence for familial aggregation was weakest for the weight-bearing joints, i.e. the knee and hip joints, as reported earlier (2,3,5). For the knee joint, environmental factors, e.g. former trauma, may play a major role in the development of ROA. Several studies of autosomal dominant hip ROA suggest a genetic defect underlying hip ROA due to dysplasia, findings of the present study suggest that such major genes contribute little to hip ROA in the general population (17,18). It should be noted that the power of the present study was most likely lower for knee and hip ROA, dichotomous traits, than for hand ROA, disk degeneration of the spine and the sum score of ROA and disk degeneration, that could be analyzed as quantitative traits.

There are several potential biases associated with our study. Selection bias might play a role when response rates were associated with ROA. Of the 368 siblings that were eligible for our study 111 refused to participate. In most instances there was no particular reason for not participating. Seven subjects indicated that they were known to have OA and did not participate for that reason. Although in general, non-response has most likely been at random, disability in elderly subjects with hip OA may have led to an underestimate of the number of hip ROA cases in siblings. Lateral knee radiographs were not available, which means no data could be presented on patellofemoral OA. Finally, heritability estimates were not calculated for men and women separately due to insufficient numbers of sibling pairs of the same sex.

It is *a priori* unlikely that environmental factors shared by siblings early in life will influence the development of ROA and disk degeneration at late middle age such that it could explain the data found in the present study. Moreover, we adjusted for possible shared factors by siblings, such as age and sex, body mass index and bone mineral density. This did not change the results of the analyses essentially and the evidence for familial aggregation of ROA and disk degeneration remained statistically significant. Furthermore, our result on hand ROA and to a lesser extent on knee ROA was comparable to the results of Spector et al in a twin study [3], in which adjustments were made for environmental factors shared by twins, by comparing monozygotic and dizygotic twin pairs. This suggests that the influence of shared environment on the familial aggregation of ROA may be very limited, which was recently supported by rejection of an environmental model in a segregation analysis of knee and hand ROA (4).

In conclusion, the present study shows a strong genetic effect for ROA and disk degeneration at multiple sites. Up to 78 percent of the total variance in ROA and disk degeneration is explained by genetic factors, independent of the (genetic) influence of body mass index and bone mineral density. In particular, a strong familial aggregation was found for hand ROA and disk degeneration of the spine. We found no evidence for a statistically significant genetic effect on the occurrence of knee and hip ROA at the population level. Previous findings suggest that there may be two possible genetic pathways, firstly the existence of a common recessive allele (frequency 0.45) and secondly a polygenic form of inheritance, perhaps in interaction with environmental factors (4). The two different mechanisms involved may explain the strong clustering of ROA in families. However, the genes underlying the familial aggregation of ROA in the population remain to be determined.

References

1. Stecher RM. Heberden's nodes. Heredity in hypertrophic arthritis of the finger joints. Am J Med Science 1941;201:801.
2. Kellgren JH, Lawrence JS, Bier F. 1963. Genetic factors in generalized osteoarthrosis. Ann Rheum Dis 1963;22:237-55.
3. Spector TD, Cicuttini F, Baker J, Loughlin J, Hart D. Genetic influences on osteoarthritis in women: a twin study. BMJ 1996;312:940-3.
4. Felson DT, Couropmitree NN, Chaisson CE, Hannan MT, Zhang Y, McAlindon TE, et al. Evidence for a mendelian gene in a segregation analysis of generalized radiographic osteoarthritis. Arthritis Rheum 1998;41:1064-71.
5. Hirsch R, Lethbridge-Cejku M, Hanson R, Scott WW, Reichle R, Plato CC, et al. Familial aggregation of osteoarthritis. Arthritis Rheum 1998;41:1227-32.

6. Burger H, van Daele PLA, Odding E, Valkenburg HA, Hofman A, Grobbee DE, et al. Osteoarthritis is associated with higher bone mineral density and increased bone loss with age: The Rotterdam Study. Arthritis Rheum 1996;39:81-6.
7. Pocock NA, Eisman JA, Hopper JC, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults. A twin study. J Clin Invest 1987;80:706-10.
8. Hofman A, Grobbee DE, De Jong PVTM, van den Ouweland FA. Determinants of disease and disability in the elderly. Eur J Epidemiol 1991;7:403-22.
9. Silman AJ, Hochberg MC. 1993. The epidemiology of the rheumatic diseases. Oxford, Oxford University Press 257-88.
10. Peyron JT, Altman R. 1992. The epidemiology of osteoarthritis. In Osteoarthritis: pathogenesis and management. Moscowitz RW, Howell DS,Goldberg VM, Mankin HJ, editors, 2nd edition. London, Saunders-Harcourt. 15-37.
11. Kellgren JH, Jeffrey MR, Ball J. (editors). 1963. The epidemiology of chronic rheumatism. Volume II: Atlas of standard radiographs of arthritis. Oxford: Blackwell Scientific Publications.
12. Haanen HCM. 1984. An epidemiological study on low-back pain. Thesis. Erasmus University Rotterdam, The Netherlands.
13. Kimura T, Nakata K, Tsumaki N, Miyamoto S, Matsui Y, Ebara S, et al. Progressive degeneration of articular cartilage and intervertebral discs. An experimental study in transgenic mice bearing a type IX collagen mutation. Int Orthopaed 1996;20:177-81.
14. Burger H, van Daele PLA, Algra D, van den Ouweland FA, Grobbee DE, Hofman A, et al. The association between age and bone mineral density in men and women aged 55 years and over: The Rotterdam Study. Bone Miner 1994;25:1-13.
15. Houwing-Duistermaat JJ, Derkx BHF, Rosendaal FR, van Houwelingen HC. Testing familial aggregation. Biometrics 1995;51:1292-301.
16. Houwing-Duistermaat JJ, van Houwelingen HC, Terhell A. Modelling the cause of dependency with application to filaria infection data. Statistics in Medic. In press.
17. Sher C, Ramesan R, Martell R, Learmoth I, Tsipouras P, Beighton P. Mild spondyloepiphyseal dysplasia (Namaqualand type): genetic linkage to the type II collagen gene COL2A1. Am J Hum Gen 1991;48:518-24.
18. Kaitila I, Korkko J, Mautinnen E, Ala-Kokko L. Phenotypic expressions of a Gly 154 Arg mutation in type II collagen in two unrelated patients with spondyloepimetaphyseal dysplasia (SEMD). Am J Med Gen 1996;63:111-22.

4

Collagen Genes and Osteoarthritis

Association of the COL2A1 gene with radiological osteoarthritis in a population-based study

The Rotterdam Study

Abstract

The authors examined the role of the COL2A1 gene in the etiology of radiographically defined osteoarthritis (ROA) in the population, using a VNTR polymorphism. Cases and referent subjects, aged 55-65 years, were derived from a prospective population based cohort study, the Rotterdam Study. The study included 532 cases with ROA in the peripheral joints, i.e. the knees, hips and hands and 282 referent subjects without ROA in these joints. The frequency distribution of alleles of the COL2A1 VNTR polymorphism in ROA cases differed from that in referent subjects, although borderline significant (p = 0.06). When stratifying for gender, women showed a statistically significant difference in allele distribution (p = 0.03), whereas in men distributions were more similar. The difference in women was explained by an increased frequency of the most common allele, 13R1. In women with ROA, carriership of one 13R1 allele was 1.71 times increased (95 percent CI 1.06-2.76), while the presence of two 13R1 alleles was 1.86 times (95 percent CI 0.95-3.64) increased as compared to referent subjects. Strongest effects were found in women with ROA in either the knee or hip joint and in women with ROA with Heberden's nodes. The present study suggests that a common allele of the COL2A1 locus plays a role in the etiology or progression of ROA in women.

Introduction

Osteoarthritis (OA) is the most common disease of the musculoskeletal system. The prevalence of OA rises from 4 percent in 18-24 year old individuals to 85 percent in subjects of ages over 74 years (1,2). The disease can lead to pain and a limited range of motion in the affected joint, and is an important cause of disability in the elderly (3). The radiographic changes in OA reflect a progressive deterioration of articular (hyaline) cartilage of diarthrodial joints with narrowing of the joint space, formation of osteophytes and development of sclerosis and pseudocystic areas in subchondral bone.

Genetic factors are involved in the rare forms of early-onset (before 30 years of age) familial OA, which have shown to be transmitted as an autosomal dominant trait (4,5). Over 40 mutations in the COL2A1 gene have indeed been identified in such families (6-8). In the 1950s, an influence of genetic factors on the etiology of OA was recognized in individuals with generalized OA, defined as OA in at least three different joint groups, in the presence of Heberden's nodes (9). Heberden's nodes are bulbous deformities at the distal interphalangeal joints of the fingers, that result from bone and soft tissue enlargement. The clinical expression of the genetic predisposition to generalized OA was found to be modified by gender and age (10-12). At the population level one recent study (13) has addressed the question of the role of genetic factors in the etiology of OA. This study of female twins suggested that genetic factors may play an important role in the occurrence of knee and hand OA in the population.

As cartilage deterioration is the hallmark of the pathologic process in OA, genes encoding collagenous (14) and non-collagenous (15) proteins of the extracellular matrix in cartilage have been proposed to be implicated in the etiology of OA. In particular, the COL2A1 gene (12q13), encoding the predominant cartilage collagen, type II, is a major candidate gene for OA. Mutations in the COL2A1 gene give rise to various grades of matrix failure in articular cartilage resulting in OA (16,17).

Findings on the role of the COL2A1 gene in OA in population studies have been controversial. An association of COL2A1 alleles and OA was found in two hospital based British studies (18,19). In contrast, an affected sibling-pair analysis (20) has failed to show a significant association with the COL2A1 locus. Neither did a study of patients with generalized OA or hand OA in the Finnish population (21). Each of these studies was of limited size and studied patients with symptomatic OA, i.e. patients visiting a clinic with joint pain. Thus the role of the COL2A1 gene in OA in the general population has not been addressed and has not been examined before in a population based study using radiological OA as outcome measurement.

We conducted an association study on the relation between the COL2A1 gene and radiographically defined OA (ROA) in a random subset of 814 subjects aged 55-65 years, derived from a single population based study (the Rotterdam Study). Cases with ROA in the peripheral joints, i.e. the knee, hip or hand joints were compared with referent subjects without ROA in the peripheral joints.

Materials and Methods

Subjects

The present study was part of the Rotterdam Study, a prospective population based cohort study of determinants and prognosis of chronic diseases in the elderly (22). For this study, all inhabitants of a suburb of Rotterdam, aged 55 years or over, including institutionalized persons, were invited to participate. In total 7983 participants were examined between 1990 and 1993. The response rate was 78 percent. From all subjects informed consent was obtained and the study was approved by the medical ethics committee of Erasmus University Medical School.

In order to ascertain ROA of relatively early onset and to target genetic predisposition to early ROA from other determinants, the current study was restricted to non-institutionalized participants aged between 55 and 65 years (n=2593). Radiographs of the peripheral joints, i.e. the knees, hips and hands, and of the thoracolumbar spine have been scored in a random subset of 944 individuals in this age category. Radiographs from other joints were not available. DNA was obtained in 814 subjects (86 percent) out of the subset of 944 individuals.

Measurements

Weight bearing anterior-posterior pelvic radiographs with both feet in 10° endorotation were obtained. Correspondingly weight bearing knee films were made with the patellae in central position. Finally, anteroposterior radiographs of the hands and wrists and lateral radiographs of the spine (Th4-S1) were obtained.

ROA was assessed by means of the Kellgren grading system (23) in five grades (0-4), using the figures and legends of the original atlas. Within the Kellgren grading system, the definition of the five grades is different for the hip joints as compared to the knee and hand joints. In the analysis definite ROA at a particular site was defined as Kellgren-score two or over in the left and/or right corresponding joint.

All radiographs were scored by two independent readers, blinded to all other data of the participant. After each set of about 150 radiographs the scores

of the two readers were evaluated. Whenever the scores differed two or more points, or, was two for one reader but one for the other, a consensus score was agreed upon. Radiographs of the knees and hips had previously been scored (24). ROA of the hand was assessed in each inter- and metacarpalphalangeal joint individually, and the first carpometacarpal and trapezoscaphoideal joints. ROA of the wrist was assessed at the radiocarpal and distal radioulnar joints. In the analysis hand ROA was defined as a Kellgren-score two or over in at least one of the 36 joints that were scored.

The presence of Heberden's nodes was determined by an examination of the hands as part of a general physical examination of the locomotor system. This was performed by trained investigators during the first visit of each participant of the Rotterdam Study to the research center. Heberden's nodes were scored in both hands separately, classified into two categories, i.e. absent or present. In the analysis definite Heberden's nodes are defined as Heberden's nodes present in the right and/or left hand.

Age, body mass index (BMI), bone mineral density (BMD) and disk degeneration of the spine were considered as possible confounders. Although disk degeneration is not considered to be ROA of the spine, these abnormalities may be associated with ROA. By definition, ROA of the spine is confined to the apophyseal joints, but these joints could not be assessed at the radiographs that were available. Disk degeneration was scored using the Kellgren grades (0-4), in which a grade zero or one denotes no or doubtful disk degeneration, a grade two denotes vertebral osteophytosis only and grades three and four vertebral osteophytosis accompanied by moderate or severe disk space narrowing. Three levels were scored, i.e. thoracic, lumbar and lumbosacral. Definite disk degeneration was defined as a Kellgren-score two or over in at least one of the three levels that were scored. Finally, bone density (g/cm^2) at the femoral neck was measured by dual energy x-ray absorptiometry as described previously (25).

VNTR polymorphism

Genotypes of the VNTR polymorphism, located 1.35 kilobases to the 3' end of the COL2A1 gene were determined in all 814 subjects of whom DNA was available. In order to genotype this multiallelic VNTR locus, PCR reactions were performed in 25 µl containing 25-50 ng genomic DNA, 2.5 pmoles of each primer (forward primer: 5'-CAA CTG ATA AAA CAG AGA GC- 3' and reverse primer: 5'-CTC CTT TGT CAT GAA CTA GC- 3' [26]), 1*Super Taq buffer, 2 µCi $\alpha[^{32}P]$-dCTP, 200 µM each of dCTP, dGTP, dTTP, and dATP, and 0.05 U of superTaq DNA polymerase (HT Biotechnology, Cambridge, UK). Amplification was initiated with 3 min denaturation at 94°C followed by 35 cycles with 93°C (1 min) denaturation, 56°C (1 min) annealing step, and a 72°C (2 min) elongation

step in a Hybaid OmniGene thermal cycler using tube control. The amplification was finished by a final incubation at 72°C for 4 min. Alleles were separated by high resolution gel electrophoresis through a denaturing 3.5 percent polyacrylamide gel, and analyzed by autoradiography. The nomenclature of alleles and allelic ladder was used from Berg and Olaisen (27).

Statistical analysis

Demographic variables were compared by using the Student's t test and chi-square test. A two step strategy of analysis was followed. Allele frequencies for cases and referent subjects were assessed by counting alleles and calculating sample proportions (with each individual contributing two alleles). Allele distributions were compared between cases and referent subjects using a likelihood ratio test (28). The rationale of this analysis is that, in a population in Hardy-Weinberg equilibrium, under the null hypothesis, the probability that one parent transmits a certain allele to the subject studied is (statistically) independent from the probability that the other parent transmits the allele. Thus, from a genetic point of view, the presence of the two alleles of an individual can be considered as two independent observations. This approach is powerful as the number of observations in N subjects is doubled to 2N alleles transmitted from the parents. A drawback is that this approach does not yield meaningful estimations for population risks. Therefore, in the second step of the analysis, the strength of the association between the COL2A1 allele(s) associated to the presence of ROA in a subject was estimated by classical epidemiological methods, i.e. multiple logistic regression. Odds ratios (ORs), derived from the logistic regression equations, are presented with 95 percent confidence intervals (CI). All analyses were carried out while adjusting for age. The analyses were additionally adjusted for disk degeneration, BMI and BMD, in order to examine whether the relationship is independent of osteophytosis and/or disk space narrowing in the spine, body mass index and bone density. Because each of the statistical tests are invalid if strata comprise less than five observations, alleles with a frequency lower then 0.05 were pooled.

Results

The baseline characteristics of our study population are shown in table 1. Out of 814 individuals 532 (65.4 percent) had ROA in the peripheral joints, i.e. the knee, hip or hand joints. These will be referred to as ROA cases. The remaining 282 (34.6 percent) individuals will be referred to as referent subjects. In these subjects ROA in the peripheral joints was absent on all radiographs assessed. ROA cases were on average 1.1 year older ($p < 0.001$) and had on average a 1.1

Table 1
Characteristics and radiographic readings from 814 subjects aged 55-65 years, Rotterdam, The Netherlands, 1990-1993

	Men		Women		All	
Total	328		486		814	
ROA cases (% of total)†	183 (55.8)		349 (71.8)		532 (65.4)	
Age (SD) in years	60.8	(2.7)*	60.7	(2.6)*	60.7	(2.6)*
Body mass index (SD) in kg/m^2	26.2	(2.9)	26.9	(4.2)*	26.7	(3.8)*
Bone mineral density femoral neck (SD) in g/cm^2	0.90	(0.12)	0.85	(0.13)**	0.87	(0.13)
Number with hand ROA (% of total)	144	(43.9)	321	(66.0)	465	(57.1)
Number with knee ROA (% of total)	42	(12.8)	106	(21.8)	148	(18.2)
Number with hip ROA (% of total)	43	(13.1)	33	(6.8)	76	(9.3)
Number with disc degeneration (% of ROA cases)	123	(67.2)	221	(63.3)*	344	(64.7)*
Number with Heberden's nodes (% of ROA cases)	32	(17.5)	108	(30.9)*	140	(26.3)*
Referent subjects = No ROA (% of total)‡	145 (44.2)		137 (28.2)		282 (34.6)	
Age (SD) in years	59.9	(2.7)	59.4	(3.0)	59.6	(2.9)
Body mass index (SD) in kg/m^2	25.7	(2.7)	25.4	(3.7)	25.6	(3.2)
Bone mineral density femoral neck (SD) in g/cm^2	0.91	(0.13)	0.83	(0.12)	0.87	(0.13)
Number with disc degeneration (% of referent subjects)	83	(57.2)	65	(47.4)	148	(52.5)
Number with Heberden's nodes (% of referent subjects)	16	(11.0)	20	(14.6)	36	(12.8)

† ROA cases had radiological osteoarthritis in the peripheral joints, e.g. the knees, hips and hands
‡ Referent subjects had no radiological osteoarthritis in the peripheral joints
* $p \leq 0.001$ (ROA cases are compared to referent subjects).
** $p = 0.03$ (ROA cases are compared to referent subjects).

Table 2
Allele frequencies of the COL2A1 VNTR polymorphism in 814 subjects aged 55-65 years, Rotterdam, The Netherlands, 1990-1993

Allele	11R1	13R1	13R2	14R1	14R2	Others	Total number of alleles	p-value
Overall	171 (0.11)	686 (0.42)	98 (0.06)	439 (0.27)	98 (0.06)	136 (0.08)	1628	
Referent subjects = No ROA	62 (0.11)	218 (0.39)	44 (0.08)	159 (0.28)	36 (0.06)	45 (0.08)	564	
ROA cases	109 (0.10)	468 (0.44)	54 (0.05)	280 (0.26)	62 (0.06)	91 (0.09)	1064	0.06
Women								
Referent subjects	32 (0.12)	100 (0.36)	24 (0.09)	76 (0.28)	16 (0.06)	26 (0.09)	274	
ROA cases	71 (0.10)	313 (0.45)	33 (0.05)	176 (0.25)	45 (0.06)	60 (0.09)	698	0.03
Hand ROA	68 (0.11)	282 (0.44)	30 (0.05)	164 (0.26)	44 (0.07)	54 (0.08)	642	0.06
Knee ROA	19 (0.09)	97 (0.46)	15 (0.07)	45 (0.21)	19 (0.09)	17 (0.08)	212	0.07
Hip ROA	8 (0.12)	31 (0.47)	2 (0.03)	15 (0.23)	3 (0.05)	7 (0.11)	66	0.25
Men								
Referent subjects	30 (0.10)	118 (0.41)	20 (0.07)	83 (0.29)	20 (0.07)	19 (0.07)	290	
ROA cases	38 (0.10)	155 (0.42)	21 (0.06)	104 (0.28)	17 (0.05)	31 (0.08)	366	0.50
Hand ROA	30 (0.10)	125 (0.43)	13 (0.05)	79 (0.27)	16 (0.06)	25 (0.09)	288	0.50
Knee ROA	6 (0.07)	34 (0.40)	4 (0.05)	27 (0.32)	4 (0.05)	9 (0.11)	84	0.50
Hip ROA	10 (0.12)	37 (0.43)	10 (0.12)	21 (0.24)	3 (0.03)	5 (0.06)	86	0.50

Numbers listed are number of alleles, with each subject contributing two alleles to the table (alleles with individual frequencies lower than 0.05 are summed in the category others). P-value is based on the likelihood ratio test by Terwilliger (28).

kg/m^2 higher ($p < 0.001$) BMI as compared to referent subjects. In women with ROA, BMD was significantly higher and the number of women with disk degeneration and with Heberden's nodes was significantly increased as compared to referent subjects. In this age category, hip ROA and disk degeneration was more frequent in men, whereas knee ROA, hand ROA and Heberden's nodes were more frequent in women.

In this study population 14 out of 23 reported alleles of the COL2A1 VNTR polymorphism were detected. In table 2, allele frequencies in all 814 genotyped subjects, as well as the allele frequencies in ROA cases and referent subjects, both overall and stratified by gender and site of ROA, are shown. In the overall analysis, the frequency distribution of alleles in ROA cases and referent subjects was different, although this difference was borderline significant ($p = 0.06$). In women the allele distribution in ROA cases as compared to referent subjects was statistically significantly different ($p = 0.03$). The difference in allele distribution between female ROA cases and referents was explained by an increased frequency of allele 13R1, while the frequencies of all other alleles, except allele 14R2, were found to be decreased or similar in cases and referent subjects. In men no significant differences were found between ROA cases and referent subjects, although the allele frequency of allele 13R1 in male cases was slightly elevated (except for male cases with knee ROA).

To estimate the strength of the association between the presence of the 13R1 allele in an individual and ROA, we performed a logistic regression analysis. We adjusted for age and other (putative) risk factors for ROA. Analyses were conducted for men and women separately. In women (table 3), the presence of one 13R1 allele (13R1 heterozygosity) in ROA cases was 1.71 times increased (95 percent CI 1.06-2.76) and the frequency of two 13R1 alleles (13R1 homozygosity) 1.86 times (95 percent CI 0.95-3.64) as compared to referent subjects. Strongest effects were found, although not significant, in the case groups of women, homozygote for allele 13R1, with either knee ROA or hip ROA (OR 2.06 with 95 percent CI 0.81-5.21 and 2.31 with 95 percent CI 0.63-8.50, respectively). In men, no significant increase in OR for ROA was found associated with the 13R1 allele (table 4). Only the OR for male cases with hand ROA tended to be increased (OR = 1.52 with 95 percent CI 0.73-3.17).

As in women Heberden's nodes were associated with ROA and have been described as part of the genetically determined forms of ROA earlier, we examined the effect of Heberden's nodes on the association between 13R1 genotypes and ROA. For this purpose, ROA cases with Heberden's nodes ($n = 140$) were compared with referent subjects without Heberden's nodes ($n = 246$). In particular in women, who were homozygote for allele 13R1, we found a strong association with ROA (table5). In female ROA cases the prevalence of 13R1 homozygotes was 3.04 times increased (95 percent CI 1.22-7.61). This effect was fur-

Table 3
Association between ROA and carriership of one or two copies of allele 13R1 in 486 women aged 55-65 years, Rotterdam, The Netherlands, 1990-1993

Genotype	13R1-/13R1-	13R1+/13R1-			13R1+/13R1+		
	n	n	OR* (95% CI)	OR† (95% CI)	n	OR* (95% CI)	OR† (95% CI)
Referent subjects = No ROA	56 Ref	62			19		
ROA cases	101 Ref	183	1.62 (1.04-2.54)	1.71 (1.06-2.76)	65	1.99 (1.07-3.70)	1.86 (0.95-3.64)
Hand ROA	96 Ref	168	1.57 (1.00-2.46)	1.68 (1.04-2.72)	57	1.77 (0.94-3.32)	1.73 (0.73-3.40)
Knee ROA	30 Ref	55	1.50 (0.83-2.72)	1.48 (0.76-2.89)	21	2.32 (1.05-5.12)	2.06 (0.81-5.21)
Hip ROA	9 Ref	17	1.60 (0.65-3.96)	1.64 (0.61-4.42)	7	2.79 (0.88-8.92)	2.31 (0.63-8.50)

13R1-/13R1- denotes individuals carrying no copy of allele 13R1, 13R1+/13R1- (13R1 heterozygotes) denotes individuals carrying one copy of allele 13R1 and 13R1+/13R1+ (13R1 homozygotes) denotes individuals carrying two copies of allele 13R1.
* OR adjusted for age, † OR adjusted for age, body mass index, bone mineral density and disk degeneration.

Table 4
Association between ROA and carriership of one or two copies of allele 13R1 in 328 men aged 55-65 years, Rotterdam, The Netherlands, 1990-1993

Genotype	13R1-/13R1-	13R1+/13R1-		13R1+/13R1+		
	n	n	OR* (95% CI)	OR† (95% CI)	n	OR* (95% CI)	OR† (95% CI)
Referent subjects = No ROA	50 Ref	72			23		
ROA cases	61 Ref	89	1.09 (0.66-1.79)	1.01 (0.60-1.69)	33	1.24 (0.64-2.41)	1.39 (0.69-2.82)
Hand ROA	48 Ref	67	1.04 (0.61-1.77)	0.94 (0.54-1.63)	29	1.40 (0.70-2.79)	1.52 (0.73-3.17)
Knee ROA	14 Ref	22	1.11 (0.51-2.39)	1.00 (0.44-2.27)	6	0.96 (0.32-2.86)	1.14 (0.36-3.57)
Hip ROA	14 Ref	21	1.08 (0.50-2.34)	0.99 (0.45-2.18)	8	1.26 (0.46-3.45)	1.19 (0.41-3.51)

13R1-/13R1- denotes individuals carrying no copy of allele 13R1, 13R1+/13R1- (13R1 heterozygotes) denotes individuals carrying one copy of allele 13R1 and 13R1+/13R1+ (13R1 homozygotes) denotes individuals carrying two copies of allele 13R1.
* OR adjusted for age, † OR adjusted for age, body mass index, bone mineral density and disk degeneration.

Table 5
Association between ROA in combination with Heberden's nodes and carriership of one or two copies of allele 13R1 in 814 subjects aged 55-65 years, Rotterdam, The Netherlands, 1990-1993

Genotype	13R1+/13R1-		13R1+/13R1+	
	OR (95% CI)*	OR (95% CI)†	OR (95% CI)*	OR (95% CI)†
ROA cases and Heberden's nodes	1.53 (0.91-2.60)	1.69 (0.97-2.94)	2.33 (1.17-4.63)	2.22 (1.06-4.62)
Men	1.31 (0.53-3.26)	1.16 (0.45-2.96)	1.29 (0.37-4.50)	1.09 (0.28-4.18)
Women	1.64 (0.87-3.12)	1.97 (1.00-3.90)	3.14 (1.33-7.42)	3.04 (1.22-7.61)
GOA	1.62 (0.73-3.59)	1.63 (0.67-3.46)	2.93 (1.06-8.07)	3.04 (1.04-8.89)
Men	1.60 (0.29-8.81)	1.41 (0.23-8.71)	1.12 (0.09-13.5)	1.21 (0.09-15.9)
Women	1.60 (0.65-3.93)	1.59 (0.62-4.09)	3.88 (1.21-12.4)	3.87 (1.13-13.3)

For this analysis referent subjects are individuals without ROA and without Heberden's nodes (n = 246). GOA denotes ROA cases with two or three joint sites affected (i.e. combinations of knee, hip and hand) in combination with the presence of Heberden's nodes (n = 47). 13R1+/13R1- denotes individuals carrying one copy of allele 13R1 and 13R1+/13R1+ denotes individuals carrying two copies of allele 13R1.
*OR adjusted for age. †OR adjusted for age, body mass index, bone mineral density and disk degeneration

ther increased in women with Heberden's nodes and generalized ROA, i.e. ROA in two or three joint sites (OR = 3.87 with 95 percent CI 1.13-13.3). In men, no substantial increase in ORs was observed (table 5).

In all logistic regression analyses, we examined whether disk degeneration in the spine, body mass index or bone mineral density of the femoral neck had an effect on these findings (see tables 3-5). Although some small differences were observed in OR or confidence interval, neither one of these analyses changed the findings materially.

Discussion

We found an association between the COL2A1 locus and ROA. A statistically significant association was observed in women, especially in those with either knee or hip ROA and those with ROA in the presence of Heberden's nodes. The association with the 13R1 allele was strongest for 13R1 homozygotes, suggesting a dose dependent effect of this allele. In women, the effect of allele 13R1 could not be explained by differences between cases and referent subjects in age, BMI, BMD or disk degeneration. In men, no significant evidence for an association between the COL2A1 locus and ROA was found.

Regarding the validity, and the comparability of our study to previous studies, the prevalence of ROA at different joint sites as observed in our study population is in keeping with earlier reports on Caucasian populations, taking into account the considerable variance in the prevalence of ROA in different populations (2). To increase the validity of ROA measurements all radiographs were scored by two trained readers. The grading system according to Kellgren (23) that was used, is a valid tool for scoring ROA in the knee and hand (29). The diagnosis of hip ROA is an issue of debate. According to Kellgren, ROA of the hip is defined as definite joint space narrowing accompanied by slight sclerosis and definite osteophytes. However, others have argued that joint space narrowing alone can be a sufficient sign for the diagnosis of hip ROA (30). Thus, the use of the Kellgren-score may have introduced misclassification, but as all radiographs were scored blind to genotyping and genotyping was performed blind to the disease status, this misclassification has most likely been non-differential. The number of alleles as well as the allele frequencies in our study population are comparable with those previously reported in Caucasian populations (26,27,31).

Case-referent studies are liable to confounding. Spurious associations may arise in genetic studies when a population consists of several subpopulations, that differ in allele and disease frequencies. This type of confounding occurs if the population studied is not homogeneous, but comprises several (ethnic) sub-

populations and is referred to as population admixture (32). To prevent this problem, it is essential that cases and referent subjects are sampled from the same source population. An advantage of the present study is that cases and referent subjects were derived from a well-defined single population, making the occurrence of bias due to admixture unlikely.

Although the present study suggests an association between the COL2A1 gene and ROA, it does not confirm the findings of two previous studies (18,19) that showed evidence for such an association. Both previous studies suggested that relatively rare clinical phenotypes of generalized OA were associated with an increased frequency of a rare allele of the COL2A1 gene. In contrast, we found common types of peripheral ROA at the knee, hip and hand to be associated with a frequent allele of the COL2A1 gene, the 13R1 allele. The only rare allele in our study that tended to be increased in ROA cases, was the 14R2 allele in women. Despite the size of the present study, the statistical power to examine differences in frequencies of rare alleles in phenotypes like generalized OA was low and therefore this study does not allow for firm conclusions on this issue.

Two previous studies (20,21) using the same VNTR polymorphism as in our study did not show an association of OA with the COL2A1 gene. Both studies were of limited size and compared a subset of relatively rare and severe OA cases with a reference group. One study (20) selected as reference group subjects with an unknown OA status, which, given the high frequency of ROA in the population, may have led to a substantial underestimation of the effects. The other (21), carried out in the Finnish population, detected only 4 out of 23 reported alleles of the COL2A1 VNTR polymorphism. The Finnish population is genetically isolated, i.e. limited number of immigrants, and relatively small. Thus, the variety in alleles is limited as compared to other populations and the allele frequencies are subject to fluctuation. It can be shown that, through this random fluctuation, (rare) alleles can disappear easily from these populations, in favor of a few predominant common alleles. Because of this process, called genetic drift, allele frequencies in the Finnish population differ significantly from those in other European countries. It is conceivable that, due to genetic drift, the COL2A1 gene does not play a role in the etiology of OA in the Finnish population, because disease associated alleles, such as the 13R1 allele, have disappeared by chance.

As to the causal inference of our findings, two issues are to be addressed. Firstly, the molecular basis for genetic association studies lies in the fact that the mutation in a disease gene that occurred in an ancestor many generations ago has been transmitted to future generations, together with parts of the chromosome directly adjacent to this disease gene. In this way, genetic markers in and surrounding the disease gene are transmitted together with the mutation in the disease gene from generation to generation until recombination occurs. It is

plausible that the COL2A1 VNTR polymorphism flags an OA related mutation within the COL2A1 gene, since this gene encodes collagen type II, the major constituent of articular cartilage. However, it can not be excluded that the VNTR polymorphism (1.35 kilobases from the COL2A1 gene) is also associated with an other gene located nearby the COL2A1 gene, and that this other gene is causally related to ROA. The Vitamin D receptor gene, located about 750 kilobases apart from the COL2A1 gene is such a gene. This gene has been found to be associated with knee ROA (33,34) and with bone density (35). However, the association between ROA and the 13R1 allele in our study is independent of bone density and therefore it is unlikely, that the Vitamin D receptor gene can explain the association between the COL2A1 gene and ROA. Another argument against the Vitamin D receptor gene explaining the observed association between the COL2A1 gene and ROA, is the finding that the Vitamin D receptor gene was not associated to Heberden's nodes (33), while the 13R1 allele of the COL2A1 gene is. A final argument is given by the, in genetic terms, large distance between the COL2A1 gene and the Vitamin D receptor gene, making the possibility that recombinations have occurred between these two genes likely. Secondly, given the cross-sectional design of our study we are unable to determine whether the observed association between the COL2A1 locus and ROA is causal. Although the mortality of OA is expected to be very limited in this age category (55-65 years), the COL2A1 gene may be involved in the progression and/or mortality of OA or mortality of associated causes of death, in stead of being a determinant of the risk of OA.

In summary, this study has shown an association between a common allele of a COL2A1 VNTR polymorphism and ROA in women. Homozygosity at the COL2A1 gene for the 13R1 allele is strongly associated to (generalized) ROA with Heberden's nodes in women. Although it has been reported earlier that the clinical expression of OA is modified by gender (10,11), the present study suggests effect modification of the association between the COL2A1 gene and ROA by gender on the level of radiographically defined OA. This is the first large population based study examining the role of the COL2A1 gene in radiographically defined OA in the peripheral joints. The effect of the COL2A1 13R1 allele appears to be dose-dependent and may contribute to the explanation of the sex- and joint-site specific patterns in the occurrence of ROA in the population.

References

1. Masi AT, Medsger TA: Epidemiology of the rheumatic diseases. In: McCarthy DJ (ed). Arthritis and allied conditions. London: Lea and Febiger, 1989;16-54.

2. Van Saase JLCM, Van Romunde LKJ, Cats A, et al. Epidemiology of osteoarthritis: Zoetermeer survey, comparison of radiological osteoarthritis in a Dutch population with that in 10 other populations. Ann Rheum Dis 1989;48:271-80.
3. Odding E, Valkenburg HA, Algra A, et al. Association of locomotor complaints and disability in the Rotterdam Study. Ann Rheum Dis 1995;54:721-5.
4. Palotie A, Ott J, Elima K, et al. Predisposition to familial osteoarthrosis linked to type II collagen gene. Lancet 1989;1:924-7.
5. Knowlton RG, Katzenstein PL, Moskowitz RW, et al. Genetic linkage of a polymorphism in the type II procollagen gene (COL2A1) to primary osteoarthritis associated with mild chondrodysplasia. New Engl J Med 1990;322:526-30.
6. Ala-Kokko L, Baldwin CT, Moskowitz RW, et al. Single base mutation in the type II procollagen gene (COL2A1) as a cause of primary osteoarthritis associated with a mild chondrodysplasia. Proc Natl Acad Sci USA 1990;87:6565-8.
7. Ahmad NN, Ala-Kokko L, Knowlton RG, et al. Stop codon in the procollagen II gene (COL2A1) in a family with the Stickler syndrome (arthro-ophthalmopathy). Proc Natl Acad Sci USA 1991;88:6624-7.
8. Williams CJ, Considine EL, Knowlton RG, et al. Spondyloepiphyseal dysplasia and precocious osteoarthritis in a family with an $Arg_{75}\rightarrow$ Cys mutation in the procollagen type II gene (COL2A1). Hum Genet 1993;92:499-505.
9. Kellgren JH, Moore R. Generalized osteoarthritis and Heberden's nodes. Br Med J 1952;1:181-7.
10. Stecher RM. Heberden Oration. Heberden's nodes. A clinical description of osteoarthritis of the finger joints. Ann Rheum Dis 1955;14:1-10.
11. Kellgren JH, Lawrence JS, Bier F. Genetic factors in generalized osteoarthrosis. Ann Rheum Dis 1963;22:237-55.
12. Cooper C, Egger P, Coggon D, et al. Generalized osteoarthritis in women: pattern of joint involvement and approaches to definition for epidemiological studies. J Rheum 1996;23:1938-42.
13. Spector TD, Cicutti F, Baker J, et al. Genetic influences on osteoarthritis in women: a twin study. BMJ 1996;312:920-4.
14. Williams CJ, Jiminez SA. Heredity, genes and osteoarthritis. Rheum Dis Clin North America 1993;19:523-43.
15. Meulenbelt I, Bijkerk C, de Wildt SCM, et al. Investigation of the association of the CRTM and CRTL1 genes with radiographically evident osteoarthritis in subjects from the Rotterdam Study. Arthritis Rheum 1997;40:1760-65
16. Eyre DR, Weis MA, Moskowitz RW. Cartilage expression of a type II collagen mutation in an inherited form of osteoarthritis associated with a mild chondrodysplasia. J Clin Invest 1991;87:357-61.
17. Kuivaniemi H, Tromp G, Prockop DJ. Mutations in collagen genes: causes of rare and some common diseases in humans. Faseb J 1991;5:2052-60.
18. Hull R, Pope FM. Osteoarthritis and cartilage collagen genes. Lancet 1989;i:1337-8.
19. Loughlin J, Irven C, Athanasou N, et al. Differential allelic expression of the type II collagen gene (COL2A1) in osteoarthritic cartilage. Am J Hum Genet 1995;56:1186-93.
20. Loughlin J, Irven C, Fergusson C, et al. Sibling pair analysis shows no linkage of generalized osteoarthritis to the loci encoding type II collagen, cartilage link protein or cartilage matrix protein. Br J Rheumatol 1994;33:1103-6.
21. Vikkula M, Nissilä M, Hirvensalo E, et al. Multiallelic polymorphism of the cartilage collagen gene: no association with osteoarthritis. Ann Rheum Dis 1993;52:762-4.
22. Hofman A, Grobbee DE, De Jong PTVM, et al. Determinants of disease and disability in the elderly. Eur J Epidemiol 1991;7:403-22.
23. Kellgren JH, Jeffrey MR, Ball J (eds). The epidemiology of chronic rheumatism. Volume II: Atlas of standard radiographs of arthritis. Oxford: Blackwell Scientific Publications, 1963.
24. Odding E. Locomotor disability in the elderly. PhD. thesis. Erasmus University Rotterdam, The Netherlands, 1994.

25. Burger H, van Daele PLA, Algra FA, et al. The association between age and bone mineral density in men and women aged 55 years and over: The Rotterdam Study. Bone Miner 1994;25:1-13.
26. Priestley L, Kumar D, Sykes B. Amplification of the COL2A1 3' variable region used for segregation analysis in a family with the Stickler syndrome. Hum Genet 1990;85:525-6.
27. Berg ES, Olaisen B. Characterization of the COL2A1 VNTR polymorphism. Genomics 1993;16:350-4.
28. Terwilliger JD. A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic marker loci. Am J Hum Genet 1995;56:777-87.
29. Spector TD, Hart DJ, Byrne J, et al. Definition of osteoarthritis of the knee for epidemiological studies. Ann Rheum Dis 1993;52:790-4.
30. Croft P, Cooper C, Wickham C, et al. Defining osteoarthritis of the hip for epidemiological studies. Am J Epidemiol 1990;132:514-22.
31. Rand S, Puers C, Skowasch K, et al. Population genetics and forensic efficiency data of 4 AMPFLP's. Int J Leg Med 1992;104:329-33.
32. Lander ES, Schork NJ. Genetic dissection in complex traits. Science 1994;265:2037-48.
33. Keen RW, Hart DJ, Lanchbury JS, et al. Association of early osteoarthritis of the knee with a Taq 1 polymorphism of the Vitamin D receptor gene. Arthritis Rheum 1997;40:1444-49.
34. Uitterlinden AG, Burger H, Huang Q, et al. Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J Clin Invest 1997;100:259-63.
35. Eisman JA. Vitamin D Receptor gene alleles and osteoporosis: an affirmative view. J Bone Miner Res 1995;10:1289-93

The COL9A1 gene and COL11A2 region and radiographically assessed osteoarthritis in subjects from a population-based study

Abstract

We performed an association study of two Short Tandem Repeat Polymorphisms (STRPs) within the COL9A1 gene and of two microsatellite marker loci flanking the COL11A2 gene and radiological osteoarthritis (ROA). Cases and referent subjects, aged 55 to 65 years, were derived from a population based cohort study, the Rotterdam Study. Cases, with ROA in the knee and/or hip joint (n = 204), were compared with age matched referent subjects free from ROA in the knees, hips and hands (n = 268). The distribution of alleles from the STRP 12B1 within the COL9A1 gene differed statistically significantly between cases and referent subjects (p = 0.002). The association between STRP 12B1 and ROA was statistically significant in women, in whom haplotype analysis suggested a protective effect of allele 12B1-A2. Neither STRP 8B2 within the COL9A1 gene, nor the microsatellites D6S291 nor TNFa, flanking the COL11A2 gene, were associated with ROA in the knee and/or hip joint. The results of our study suggest that the COL9A1 gene may contribute to the pathogenesis of osteoarthritis in women. We found no evidence for a role of the COL11A2 region or for the TNF-α gene in the occurrence of osteoarthritis.

Introduction

Osteoarthritis (OA) is a highly prevalent complex disease, characterized by a progressive degeneration of articular (hyaline) cartilage. The pathophysiologic changes can radiographically be observed as osteophyte formation at the joint margins, joint space narrowing, subchondral sclerosis of bone and formation of bony cysts. Although these radiographically assessed abnormalities do not ubiquitously lead to clinical OA (1,2), i.e. pain, stiffness or limited range of motion of the affected joint, they reflect cartilage degeneration, which can be considered as the pathophysiologic hallmark underlying clinical OA.

The extracellular matrix of articular cartilage consists of proteoglycan aggregates embedded in a network of collagen fibrils, containing collagen's type II, IX and XI (3). Although type IX collagen constitutes not more than 1% of the total collagen in articular cartilage, it is important in stabilizing the articular collagen network, mainly consisting of type II collagen fibrils (95% of total collagen in cartilage) (4-6). Type IX collagen is a heterotrimeric protein, encoded by three different genes (3). The COL9A1 gene, encoding the α1(IX) chain, is located on chromosome 6 (6q12-q13) (7). The absence of the α1(IX) chain or the synthesis of a shortened α1(IX) chain in mice, leads to degeneration of articular cartilage similar to that in human OA (8,9). It has been suggested that dysfunctional type IX collagen alters the mechanical properties of the collagen network in articular cartilage, leading to joint cartilage more prone to damage from mechanical stress (8). However, up to date there is no empirical evidence that the COL9A1 gene is involved in OA in humans. Mutations in the COL9A2 gene induce severe phenotypes with multiple epiphyseal dysplasia that are very different from common forms of OA in the elderly (10). For the COL9A3 gene mutations or hereditary diseases have not been reported.

The fibrillar collagen type XI is also a constituent of cartilage collagen and is closely associated with collagen type II. It is important in regulating the diameter of the type II collagen fibril (11) and has been shown to be important for the integrity and proper development of the skeleton (12). Furthermore, type XI collagen binds with high affinity to proteoglycans, which may be important for anchoring cartilage proteoglycans to the collagen fibrillar network (13). It consists of three distinct α chains of which the α2(XI) chain is transcribed from the COL11A2 gene, which has been localized on chromosome 6 (6p21) within the major histocompatibility complex (14). A dominant as well as a recessive mutation in the COL11A2 gene has been described, both leading to spondyloepiphyseal dysplasia associated with severe OA (12). Other mutations or polymorphisms in the COL11A2 gene may give rise to cartilage pathology in the general population. Data on the COL11A1 gene suggest that this gene plays a role in

skeletal morphogenesis (15). Mutations in this gene lead to severe phenotypes, e.g. Stickler syndrome (16). The third α chain of type XI collagen is encoded by the COL2A1 gene, which has extensively been studied in human OA.

We aimed to study collagen genes with the largest possibility of playing a role in the occurrence of common forms of OA in the elderly, i.e. the COL2A1, COL9A1 and COL11A2 genes. Data on the COL2A1 gene has been presented previously (unpublished data). In this paper we studied the associations between the COL9A1 gene and COL11A2 region and radiological osteoarthritis (ROA), in a subset of 472 subjects aged 55-65 years, derived from a single population based study. Cases with ROA in the knee and/or hip joints were compared with referent subjects without ROA in the peripheral joints, i.e. the knee, hip and hand joints.

Material and Methods

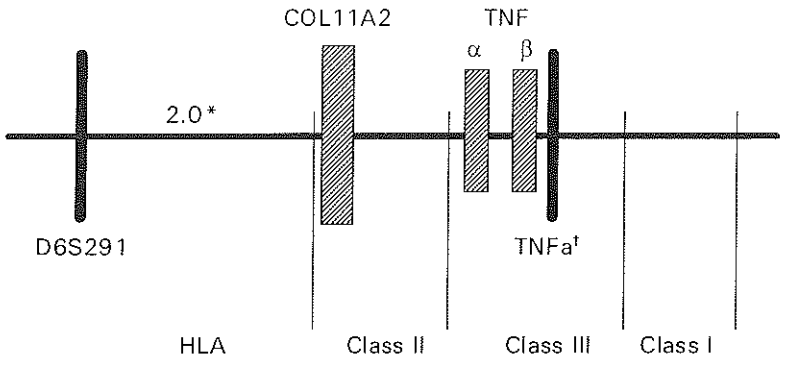
Subjects

Cases and referent subjects were derived from a prospective population based follow-up study of determinants and prognosis of chronic diseases in the elderly (the Rotterdam Study) (17). All inhabitants of a suburb of Rotterdam aged 55 years or over were invited to participate. In total 7983 participants were examined. The response rate was 78 percent. The medical ethics committee of Erasmus University Medical School has approved the study and written informed consent was obtained from all participants.

In order to ascertain ROA of relatively early onset, the present study was limited to participants aged between 55 and 65 years (n=2593). Radiographs of the peripheral joints, i.e. the knees, hips and hands, have been scored in a random subset of 944 individuals in this age category. Less than one percent of the 944 participants were related in the first degree. On the basis of the radiographic data, we maximized the phenotypic contrast between cases and referent subjects. Cases were defined as subjects with ROA in at least one of the large weight bearing joints, i.e. the knee and hip joints (n = 216), whereas referent subjects were defined as subjects without ROA in the peripheral joints, i.e. the knees, hips and hands (n = 332). The referent group corresponds roughly with the lower 30% of the distribution of ROA in the population. DNA was obtained in 472 subjects (86 percent) out of this subset of 548 cases and referent subjects.

Measurements

Weight bearing anterior-posterior radiographs of the hips and knees and anterior-posterior radiographs of the hands were obtained. ROA was assessed by



* Distance in cM between marker D6S291 and the COL11A2 gene.
† Marker TNFa is located 3.5 kb telomeric to the TNF-β gene and 1.5 cM telomeric to the COL11A2 gene.

Figure 1
Schematic representation of the position of the flanking markers D6S291 and TNFa to the COL11A2 gene.

means of Kellgren's grading system (18) in five grades (0-4), using the figures and legends of the original atlas. In the analysis, definite ROA was defined as a Kellgren-score two or over. Two independent readers, blinded to all other data, scored all radiographs. Whenever the scores were different by two or more points, or, was two for one reader but one for the other, a consensus score was agreed upon. Radiographs of the knees and hips had been scored previously (1). ROA of the hand was assessed in each inter- and metacarpalphalangeal joint, and the first carpometacarpal, trapezoscaphoideal, radionavicular and distal radioulnar joints. In the analysis, hand ROA was defined as Kellgren-score two or over in at least one of the 18 joints in either hand that were scored.

Genotyping

COL9A1 gene

Two Short Tandem Repeat Polymorphisms (STRP), 509-8B2 and 509-12B1 within the COL9A1 gene were used. PCR reactions were performed in a total volume of 10 µl, containing 10-15 ng genomic DNA and 1pM of each primer as described by Warman et al (19). Amplification was initiated with 3 min denaturation at 93°C followed by 35 cycles of 95°C for 15s, 52°C for 30s, and 72°C for 30s, with a final incubation at 72°C for 4 min. The nomenclature of alleles and allelic ladder was used from Warman et al (19).

COL11A2 region

No informative markers within the COL11A2 gene were available, therefore we used the polymorphic flanking markers D6S291 and TNFa (figure 1). The TNF locus, consisting of the TNF-α and -β genes, is located within the HLA class III region, 250 kb telomeric to HLA-B (class I) and 340 kb centromeric to the C2/BF complex (class III). The TNF-α gene itself is also a candidate gene for OA as a modulator of chondrocyte function (20). PCR reactions were performed in a total volume of 25 µl containing 33 ng genomic DNA, 2.5pM of each primer and 0.05 U superTAq DNA polymerase. Amplification was initiated with 3 min denaturation at 93°C followed by 35 cycles at 93°C for 30s, at 60°C for 30s, and at 72°C for 30s, with a final incubation at 72°C for 4 min. The nomenclature of alleles and allelic ladder was used from Weissenbach (21) for D6S291 and from Nedospasov (22) for TNFa.

For all markers allelic bands were separated on a 6.0 percent denaturing polyacrylamide gel and analyzed by autoradiography.

Statistical analysis

Using Student's t test and chi-square test demographic variables were compared. Allele frequencies in cases and referent subjects were assessed through counting alleles and calculating sample proportions. For each marker, Hardy-Weinberg equilibrium (HWE) tests were performed using the exact test specifically developed for this purpose by Guo and Thompson (23). When a case or referent group was found to be in Hardy-Weinberg equilibrium, allele distributions were compared between cases and referent subjects with an exact test. We subsequently tested for genotypic disequilibrium between markers 8B2 and 12B1 at

Table 1
Characteristics of the study population.

	Men		Women		All	
Cases = knee and/or hip ROA (%)	79	(39)	125	(61)	204	(100)
Age (SD) in years	60.8	(2.6)	60.8	(2.5)	60.8	(2.5)
Number with knee ROA (%)	41	(28)	106	(72)	147	(100)
Number with hip ROA (%)	43	(57)	33	(43)	76	(100)
Referent subjects = No ROA (%)	137	(51)	131	(49)	268	(100)
Age (SD) in years	60.0	(2.7)	59.4	(3.0)	59.7	(2.8)

ROA = Radiological osteoarthritis. ROA at a particular joint site is defined as Kellgren-score two or over in the right and/or left corresponding joint.

the COL9A1 gene and markers D6S291 and TNFa at the COL11A2 region. HWE test, test for population differentiation and test for genotypic disequilibrium were conducted, using the Genepop statistical package (24). These tests are based on the Markov chain method, which yields exact p-values. Furthermore, an expectation maximization (EM) algorithm was used to obtain estimated haplotype frequencies and a chi-square test was used to compare haplotype frequencies between cases and referent subjects (25).

Results

The characteristics of our study population are shown in table 1. Out of 204 cases 147 subjects had knee ROA (106 women and 41 men) and 76 subjects had hip ROA (33 women and 43 men). In the referent group, consisting of subjects without ROA in the knees, hips, or hands, men and women were equally distributed. On average, cases were 1.1 years older than referent subjects.

COL9A1 gene

Genotype and allele analysis

We detected 12 alleles of the COL9A1 STRP 8B2 and 10 alleles of the STRP 12B1, which means in both cases two additional alleles as compared to Warman et al (19). In table 2, allele frequencies of 268 referent subjects and 199 cases (genotyping was unsuccessful in five cases) of STRPs 12B1 and 8B2 are shown. In table 3, the allele frequencies are shown for men and women separately. Hardy-Weinberg equilibrium testing showed that for both microsatellite marker loci cases as well as referent subjects were in equilibrium. This did not alter when stratifying cases and referent subjects by gender (table 3). The frequency distribution of alleles of the STRP 12B1 differed statistically significantly between cases and referent subjects ($p = 0.002$) (see table 2). An increased frequency of allele A5 and decreased frequency of allele A2 in cases as compared to referent subjects was observed in both men and women, though this was statistically significant ($p < 0.05$) in women only (see table 3). No differences were observed between cases with knee ROA and cases with hip ROA (data not shown). The frequency distribution of alleles of the STRP 8B2 was neither overall ($p = 0.20$) nor in men or women separately statistically significantly different in cases and referent subjects.

LD and haplotype analysis

Linkage disequilibrium (LD) analysis showed that overall in cases and referent subjects no evidence existed for LD between the STRPs 12B1 and 8B2 (referent subjects $p = 0.36$ and cases $p = 0.29$). However, in the gender specific analyses,

Table 2
Allele frequencies COL9A1 12B1 and 8B2 in cases (knee and/or hip ROA) and referents (free from ROA in the knee, hip and hand)

STRP 12B1 Allele	A2	A3	A4	A5	A6	A7	A8	Others	Total	HWE	PD
Referent subjects	96 (0.18)	30 (0.06)	171 (0.32)	67 (0.13)	44 (0.08)	33 (0.06)	91 (0.17)	4 (0.01)	536	0.32	
Cases	39 (0.10)	14 (0.04)	137 (0.34)	76 (0.19)	36 (0.09)	19 (0.05)	72 (0.18)	5 (0.01)	398	0.68	0.002
STRP 8B2 Allele	A4	A5	A6	A7	A8	A9		Others	Total	HWE	PD
Referent subjects	21 (0.04)	231 (0.43)	140 (0.26)	29 (0.05)	22 (0.04)	62 (0.12)		31 (0.06)	536	0.76	
Cases	20 (0.05)	159 (0.40)	86 (0.22)	33 (0.08)	24 (0.06)	49 (0.12)		27 (0.07)	398	0.68	0.20

Alleles with frequencies lower than 0.03 are summed in the category others. HWE = p-value Hardy Weinberg equilibrium test. PD = p-value comparing the allele distributions between cases and referent subjects (population differentiation).

Table 3
Allele frequencies COL9A1 12B1 and 8B2 in cases (knee and/or hip ROA) and referents (free from ROA in the knee, hip and hand), stratified by gender.

STRP 12B1 Allele	A2	A3	A4	A5	A6	A7	A8	Others	Total	HWE	PD
Referents women	43 (0.16)	16 (0.06)	77 (0.29)	36 (0.14)	21 (0.08)	17 (0.06)	51 (0.19)	1 (0.01)	262	0.53	
Cases women	19 (0.08)	9 (0.04)	83 (0.34)	47 (0.19)	20 (0.08)	11 (0.05)	51 (0.21)	4 (0.02)	244	0.44	0.04
Referents men	53 (0.19)	14 (0.05)	94 (0.34)	31 (0.11)	23 (0.08)	16 (0.06)	40 (0.15)	3 (0.01)	274	0.30	
Cases men	20 (0.13)	5 (0.03)	54 (0.35)	29 (0.19)	16 (0.10)	8 (0.05)	21 (0.14)	1 (0.01)	154	0.84	0.21

STRP 8B2 Allele	A4	A5	A6	A7	A8	A9	Others	Total	HWE	PD
Referents women	10 (0.04)	110 (0.42)	72 (0.27)	15 (0.06)	11 (0.04)	30 (0.11)	14 (0.05)	262	0.68	
Cases women	14 (0.06)	97 (0.39)	56 (0.23)	22 (0.09)	14 (0.06)	27 (0.11)	14 (0.06)	244	0.60	0.67
Referents men	11 (0.04)	121 (0.44)	68 (0.25)	14 (0.05)	11 (0.04)	32 (0.12)	17 (0.06)	274	0.98	
Cases men	6 (0.04)	62 (0.40)	30 (0.19)	11 (0.07)	10 (0.06)	22 (0.14)	13 (0.08)	154	0.49	0.44

Alleles with frequencies lower than 0.03 are summed in the category others. HWE = p-value Hardy Weinberg equilibrium test. PD = p-value comparing the allele distributions between cases and referent subjects (population differentiation).

Table 4
Allele frequencies of flanking markers D6S291 and TNFa of the COL11A2 gene in cases (knee and/or hip ROA) and referents (no ROA).

D6S291 Allele	A3	A4	A5	A6	A7	A8	Others	Total	HWE	PD
Referent subjects	30 (0.08)	19 (0.05)	21 (0.05)	25 (0.06)	182 (0.47)	108 (0.28)	3 (0.01)	388	0.13	
Cases	40 (0.10)	15 (0.04)	30 (0.08)	22 (0.06)	172 (0.43)	118 (0.30)	3 (0.01)	400	0.44	0.73

TNFa Allele	A4	A5	A8	A9	A10	A11	A13	Others	Total	HWE	PD
Referent subjects	73 (0.19)	47 (0.12)	24 (0.06)	36 (0.09)	32 (0.08)	19 (0.05)	124 (0.32)	33 (0.08)	388	0.14	
Cases	58 (0.14)	45 (0.11)	27 (0.07)	40 (0.10)	26 (0.06)	45 (0.11)	122 (0.30)	37 (0.09)	400	0.62	0.22

Alleles with frequencies lower than 0.03 are summed in the category others. HWE = p-value Hardy Weinberg equilibrium test. PD = p-value comparing the allele distributions between cases and referent subjects (population differentiation).

we observed LD in the female case group (p = 0.02). Neither in male cases nor in male or female referent subjects LD was observed. We subsequently investigated whether in women specific haplotypes were responsible for the associations observed between ROA and alleles of STRP 12B1. These analyses were based on the estimated haplotype frequencies derived from an EM algorithm. The occurrence of haplotypes 12B1-A2/8B2-A5 and 12B1-A2/8B2-A6 was respectively 3.2 times (adjusted p-value = 0.01) and 7.1 times (adjusted p-value < 0.002) decreased in female cases as compared to female referent subjects. The frequencies of both 8B2 alleles, A5 and A6, were decreased in cases as compared to referent subjects in the overall (table 2) and gender specific (table 3) analyses, although these differences were not statistically significant. Allele 12B1-A5 forms haplotypes with all alleles of STRP 8B2. No specific haplotype with the allele 12B1-A5 was observed with a statistically significantly increased frequency in cases as compared to referent subjects.

COL11A2 region

Of the microsatellite marker locus D6S291 8 alleles were detected, while for TNFa 14 different alleles were observed. In table 4, allele frequencies of 194 referent subjects and 200 cases (genotyping was unsuccessful in four cases) of microsatellites D6S291 and TNFa are shown. Genotypes of both dinucleotide repeats were in Hardy-Weinberg equilibrium in cases and referent subjects (see table 4). No significant differences were found in allele distributions between cases and referent subjects for D6S291 or TNFa, neither in the overall analysis (table 4) nor in the analyses stratified according to gender (data not shown). Also, LD analysis failed to show significant evidence for an association between the COL11A2 region and ROA.

Discussion

We found a statistically significant difference in the allele distribution between cases with ROA in the knee and/or hip joint and referent subjects without ROA for the STRP 12B1 of the COL9A1 gene. This difference was mainly due to the lower frequency of allele A2 and higher frequency of allele A5 in cases as compared to referent subjects. The association between STRP 12B1 and ROA was strongest in women. Linkage disequilibrium was found in the female case group, in whom subsequent haplotype analysis led to a refinement of the association of ROA with 12B1-A2/8B2-A5 and 12B1-A2/8B2-A6, suggesting a protective effect of these haplotypes. Allelic and haplotype distributions of the microsatellite marker loci D6S291 and TNFa, flanking the COL11A2 gene, were not different in cases as compared to referent subjects.

The allele frequencies in our study population are similar to those previously reported in Caucasian populations (19,21,22). All radiographs were scored before genotyping and genotyping was performed blind to the disease status. Therefore, misclassification will most likely be random and is not likely to be the cause of the associations found. In the present study cases and referent subjects were derived from a homogeneous Caucasian population, making bias due to population admixture unlikely (26).

STRPs 12B1 and 8B2 are both located within the COL9A1 gene, but linkage disequilibrium was found in the female case group only. This suggests that the frequency of recombination within the COL9A1 gene is likely to be high and may be different in this region for men and women. Gender specific recombination rates have previously been found for other region's (27). Interestingly, the association reported in this paper between the COL9A1 gene and ROA was strongest for women, in particular for the 12B1 allele A2 and specifically for haplotypes 12B1-A2/8B2-A5 and 12B1-A2/8B2-A6. The effect of 12B1 allele A5, which frequency is increased in cases as compared to referent subjects, was not confirmed by haplotype analysis. In women, STRP 8B2 itself was not significantly associated with ROA, despite the fact that linkage disequilibrium was found with STRP 12B1 in the female case group. This can be explained by the relatively high frequencies of the A5 and A6 alleles of STRP 8B2. These alleles occur therefore on many other haplotypes than the ones involved in the linkage disequilibrium with allele A2 of STRP 12B1, with which allele an association with ROA was detected.

Our findings did not show evidence for a role of the COL11A2 region in the occurrence of ROA. Keeping in mind the relatively large distance between the COL11A2 gene and the two flanking microsatellite marker loci D6S291 and TNFa, in particular the haplotype analysis decreases the chance that the COL11A2 gene itself plays an important role in the occurrence of ROA in the general population. However, this finding needs to be confirmed in another study. Both TNFa and the COL11A2 gene are located within the HLA region, which region has a low frequency of recombination and therefore TNFa can serve as a reliable marker locus for the COL11A2 gene. TNF-α gene may be considered a candidate gene for OA (20), but was not associated with ROA in our study. This minimizes the possibility that the TNF-α gene plays a role in the occurrence of ROA in the general population.

Although present in small amounts, type IX collagen is an important constituent of normal articular cartilage. It stabilizes and lends cohesion to the collagen network (28). Differences in the gene products encoding type IX collagen may lead to the synthesis of abnormally assembled type IX collagen fibrils, that are more prone to damage from repetitive and long-term mechanical stress. This

has indeed been shown in transgenic mice, in which the synthesis of shortened α1(IX) chains gives rise to osteoarthritic changes in knee joints (8).

The present study shows a significant association between the STRP 12B1 of the COL9A1 gene and ROA in the general population. Following the studies in transgenic mice, our population-based findings emphasize the COL9A1 gene as an interesting candidate gene for human OA.

References

1. Odding E: Locomotor disability in the elderly. An epidemiological study of its occurrence and determinants in a general population of 55 years and over. PhD thesis, Erasmus University Rotterdam, The Netherlands, 1994.
2. O'Reilly SC, Muir KR, Doherty M: Screening for pain in knee osteoarthritis: which question? Ann Rheum Dis 1996; 55: 931-933.
3. Bruckner P, Van der Rest M: Structure and function of cartilage collagens. Micr Res & Tech 1994; 28: 378-384.
4. Wu JJ, Woods PE, Eyre DR: Identification of cross-linking sites in bovine cartilage type IX collagen reveals an antiparallel type II-type IX molecular relationship and type IX to type IX bonding. J Biol Chem 1992; 267: 23007-23014.
5. Diab M: The role of type IX collagen in osteoarthritis and rheumatoid arthritis. Orth Review 1993; 26: 165-170.
6. Diab M, Wu JJ, Eyre DR: Collagen type IX from human cartilage: a structural profile of intermolecular cross-linking sites. Biochem J 1996; 314: 327-332.
7. Kimura T, Mattei MG, Stevens JW, Goldring MB, Ninomiya Y, Olsen BR: Molecular cloning of rat and human type IX collagen cDNA and localization of the alpha1(IX) gene on the human chromosome 6. Eur J Biochem 1989; 179: 71-78.
8. Nakata K, Ono K, Miyazaki JI, Olsen BR, Muragaki Y, Adachi E, Yamamura KI, Kimura T: Osteoarthritis associated with mild chondrodysplasia in transgenic mice expressing α1(IX) collagen chains with a central deletion. Proc Natl Acad Sci USA 1993; 90: 2870-2874.
9. Fässler R, Schnegelsberg PNJ, Dausman J, Shinya T, Muragaki Y, McCarthy MT, Olsen BR, Jaenisch R: Mice lacking α1(IX) collagen develop noninflammatory degenerative joint disease. Proc Natl Acad Sci USA 1994; 91: 5070-5074.
10. Muragaki Y, Mariman ECM, van Beersum SEC, Perälä M, van Mourik JB, Warman ML, Olson BR, Hamel BCJ: A mutation in the gene encoding the α2 chain of the fibril associated collagen IX, COL9A2, causes multiple epiphyseal dysplasia (EDM2). Nature Genet 1996; 8: 103-105.
11. Thom JR, Morris NP: Biosynthesis and proteolytic processing of type XI collagen in embryonic chick sterna. J Biol Chem 1991; 266: 7262-7269.
12. Vikkula M, Mariman ECM, Lui VC, Zhidkova N, Tiller G, Goldring M, van Beersum S, de Waal Malefijt M, van den Hoogen F, Ropers HH, Mayne R, Cheah KS, Olsen BR, Warman ML, Brunner HG: Autosomal dominant and recessive osteochondrodysplasias associated with the COL11A2 locus. Cell 1995; 80: 431-437.
13. mith GN, Williams JM, Brandt KD: Interaction of proteoglycans with the pericellular (1α, 2α, 3α) collagens of cartilage. J Biol Chem 1985; 260: 10761-10767.
14. Lui VC, Ng LJ, Sat EWY, Cheah KS: The human α2(XI) collagen gene (COL11A2): completion of coding information, identification of the promoter sequence, and pre-

cise localization within the major histocompatibility complex reveal overlap with the KE5 gene. Genomics 1996; 32: 401-412.
15. Li Y, Lacerda DA, Warman ML, Beier DR, Yoshioka H, Ninomiya Y, Oxford JT, Morris NP, Andrikopoulos K, Ramirez F, Wardell BB, Lifferth GD, Teuscher C, Woodward SR, Taylor BA, Seegmiller RE, Olsen BR: A fibrillar collagen gene, Col11a1, is essential for skeletal morphogenesis. Cell 1995; 80: 423-430.
16. Richards AJ, Yates JRW, Williams R, Payne SJ, Pope FM, Scott JD, Snead MP: A family with Stickler syndrome type 2 has a mutation in the COL11A1 gene resulting in the substitution of glycine 97 by valine in α1(XI) collagen. Hum Mol Genet 1996; 5: 1339-1343.
17. Hofman A, Grobbee DE, De Jong PTVM, Van den Ouweland FA: Determinants of disease and disability in the elderly. Eur J Epidemiol 1991; 7: 403-422.
18. Kellgren JH, Jeffrey MR, Ball J (eds): The epidemiology of chronic rheumatism. Volume II: Atlas of standard radiographs of arthritis. Oxford: Blackwell Scientific Publications, 1963.
19. Warman ML, Tiller GE, Polumbo PA, Seldin MF, Rochelle JM, Knoll JHM, Cheng SD, Olsen BR: Physical and linkage mapping of the human and murine genes for the α1 chain of type IX collagen (COL9A1). Genomics 1993; 17: 694-698.
20. Saklatvala J: Tumor necrosis factor α stimulates resorption and inhibits synthesis of proteoglycan in cartilage. Nature 1986; 322: 547-549.
21. Weissenbach J, Gyapay G, Dib C, Vignal A, Morisette J, Millasseau P, Vaysseix G, Lathrop M: A second generation linkage map of the human genome. Nature 1992; 359: 794-801.
22. Nedospasov SA, Udalova IA, Kuprash DV, Turetskaya RL: DNA sequence polymorphism at the human tumor necrosis factor (TNF) locus. J Immunol 1991; 147: 1053-1059.
23. Guo SW, Thompson EA: Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 1992; 48: 361-372.
24. Raymond M, Rousset F: Genepop (version 1.2): population genetics software for exact tests and ecumenicism. J Heredity 1995; 86: 248-249.
25. Schneider S, Kueffer JM, Roessli D, Excoffier L: Arlequin ver 1.1: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland, 1997.
26. Lander ES, Schork NJ: Genetic dissection of complex traits. Science 1994; 265: 2037-2048.
27. Paldi A, Gyapay G, Jami J: Imprinted chromosomal regions of the human genome display sex-specific meiotic recombination frequencies. Current Biology 1995; 5: 1030-1035.
28. Eyre DR: Structure and function of the cartilage collagens: role of type IX collagen in articular cartilage. In: Brandt KD (ed) Cartilage changes in osteoarthritis, Indiana University School of Medicine, Indianapolis, pp 12-16, 1990.

A sibling pair study on the role of the COL2A1 and COL9A1 genes in radiological osteoarthritis

Abstract

Objective *To investigate the role of the COL2A1 and COL9A1 genes in the occurrence of radiological osteoarthritis (ROA) and/or disk degeneration through a sibling pair approach.*

Methods *Probands (n = 118) were derived from a random sample of a population-based cohort study (the Rotterdam Study) and were affected with ROA and/or disk degeneration at multiple joint sites. All available siblings that were willing to participate were recruited (n = 257). ROA in the knees, hips, and hands and disk degeneration of the spine was scored according to the Kellgren grading system. ROA was considered a quantitative trait locus, using a sum score of ROA and disk degeneration, a sum score of hand ROA, and a sum score of disk degeneration. Analyses were performed using MAPMAKER/SIBS.*

Results *For the COL2A1 locus, a reduction was observed in the variance for the sum score of ROA and disk degeneration and for the sum score of hand ROA (quantitative trait loci), with an increase in the number of alleles shared between female siblings (not statistically significant). Effects were stronger for women with ROA and Heberden's nodes and for*

women with generalized ROA. For the COL9A1 locus, no evidence for linkage was found between the three phenotypes studied and this locus.
Conclusion *Findings of the present study are compatible with a role of the COL2A1 locus in the occurrence of ROA in the peripheral joints.*

Introduction

Osteoarthritis (OA) is a disease characterized by degeneration of articular cartilage and formation of osteophytes at the joint margins. Recent population-based studies have demonstrated a considerable genetic component in the etiology of common forms of OA (1-3). High sib-sib correlations were obtained in these studies when OA was regarded a quantitative trait, by summing all joints in the hands and knees affected with radiological OA (ROA). Which genes are responsible for this genetic influence in OA remains largely to be determined, although several candidate genes have been proposed that could explain part of this genetic susceptibility (4-8). Among others these are genes encoding cartilage specific collagens, growth factors, metalloproteases and non-collagenous cartilage proteins.

For the COL2A1 gene, encoding the major cartilage protein, collagen type II, findings have been controversial. Two British hospital based studies showed an association between rare alleles of the COL2A1 gene and radiological OA (ROA) at multiple sites (4,5). Association studies in the Finnish population and Belgian population (postmenopausal women) and a sibling pair study of limited size in the British population failed to show an association with the COL2A1 locus (9-11). We found an association between the COL2A1 locus and ROA in women, in a population-based study (see Chapter 4.1). Strongest effects were found for women with generalized OA and for women with Heberden's nodes.

The COL9A1 gene encodes the α1 chain of the heterotrimeric protein collagen type IX, which is covalently cross linked to collagen type II fibrils and stabilizes the articular collagen network (12). We recently performed the first association study on ROA and the COL9A1 gene and found evidence for a protective effect of this locus on the occurrence of ROA, especially in women (see Chapter 4.2).

Our findings in both association studies need to be interpreted with caution, as these studies can easily give rise to a false positive result (13). We have conducted a sibling pair study in 257 siblings of 118 probands with ROA and disk degeneration at multiple joint sites. We examined the genes, i.e. the COL2A1 and COL9A1 genes, of which we previously found an association with ROA. ROA was considered a quantitative trait based on the number of joints in the knees, hips, hands, and spine affected with ROA or disk degeneration.

Materials and Methods

Subjects

Probands were derived from a prospective population-based follow-up study of determinants and prognosis of chronic diseases in the elderly, the Rotterdam Study (14). In this study all persons living in Ommoord, a suburb of Rotterdam, aged 55 years and over were invited to participate. In total 7983 participants (response rate of 78 percent) were examined. The medical ethics committee of Erasmus University Medical School has approved the study and written informed consent was obtained from all participants.

A random sample of 944 non-institutionalized individuals aged between 55 and 65 years was drawn from the Rotterdam Study. In all individuals, radiographs of the peripheral joints, i.e. knees, hips and hands, and of the thoracolumbar spine were scored for respectively ROA and disk degeneration. Probands for the sibling pair study had to have two or more of these four joint sites affected in order to be selected. In case individuals had hand ROA and disk degeneration of the spine, which is the most common combination present, they had to have additional Heberden's nodes to be included as probands. The 221 probands that were willing to participate (response rate 88 percent), had a total of 708 siblings born alive, of which 368 siblings were eligible to our study. We were able to recruit 257 siblings (70 percent), whom were all examined at the research center.

Measurements

For all individuals the following radiographs were obtained: weightbearing anterior-posterior pelvic radiographs with both feet in 10° endorotation, weightbearing knee radiographs with the patellae in central position, anteroposterior radiographs of the hands and wrists and lateral radiographs of the spine (Th4-S1).

The use of radiologically defined changes, e.g. osteophyte formation and joint space narrowing, is widely accepted in epidemiological research concerning OA (15). ROA of the spine could not be assessed on the lateral radiographs that were made. Instead, disk degeneration of the spine was scored, which may share a common genetic etiology with ROA of the peripheral joints (16). ROA of the peripheral joints and disk degeneration of the spine were assessed by means of the Kellgren grading system in five grades (0-4), using the figures and legends of the original atlas (17). Two independent readers, who had no knowledge of the other data of the participant, scored all radiographs. After each set of about 150 radiographs the scores of the two readers were evaluated. Whenever the scores were two or more points different, or, was two for one reader but one for

the other, a consensus score was agreed upon. For each individual joint that was scored, definite ROA and definite disk degeneration was present whenever the Kellgren-score was two or over.

The total number of joints that was scored amounted to 23, consisting of the right and left knee and hip joints, 16 joints in the hands (right and left separate) and three levels in the spine, i.e. thoracic, lumbar and lumbosacral. These 23 joints constituted a sum score of ROA and disk degeneration, which summarizes the pathophysiologic process of cartilage degeneration, the hallmark of OA, as a quantitative trait. Each one of the 23 joints contributed one point to the sum score in case the Kellgren-score in this joint was two or over.

The presence of Heberden's nodes was determined by an examination of the hands, which was performed by trained investigators at the research center. Heberden's nodes are bulbous deformities at the distal interphalangeal joints of the fingers that result from bone and soft tissue enlargement. They were classified as absent or present, without knowledge of the radiographic findings.

Genotyping

Genotypes of a VNTR polymorphism, located 1.35 kb to the 3' end of the COL2A1 gene, were determined, following procedures and using an allelic ladders as described previously (18). In total, 15 alleles of the COL2A1 VNTR polymorphism were detected. For the COL9A1 gene, Short Tandem Repeat Polymorphisms (STRP) 509-8B2 and 509-12B1 were used according to conditions and using nomenclature of alleles as described previously (19). In this study population, 15 and 10 alleles were detected for respectively STRP 8B2 and STRP 12B1. All genotyping was performed without knowledge of any other data of the participant.

Statistical analysis

In the analyses OA was considered a quantitative trait locus (QTL) that has the advantage that no sibling pairs are lost for data analysis and which is the most powerful approach, statistically, for a common trait. In order to analyze OA as a QTL three phenotypes were used. Firstly, the sum score of ROA and disk degeneration, which is a variable with a range from 0 to 23. Secondly, a score summing the number of joints affected in both hands, with a range from 0 to 16. Thirdly, a score summing the number of levels affected with disk degeneration in the spine, which has a range from 0 to 3. The QTL analyses were performed for all pairs, for female pairs, and for male pairs. Other subgroup analyses included pedigrees of probands with Heberden's nodes and pedigrees of probands carrying the 13R1 allele of the COL2A1 VNTR polymorphism. We previ-

ously found an association between this allele and ROA in women (see Chapter 4.1).

All sibling pair analyses were performed using MAPMAKER/SIBS (20), which infers the full probability distribution of the identical by decent (IBD) status. For the QTL analyses both a maximum likelihood (ML) method and a nonparametric (NP) method were used. The ML method renders the variance of the quantitative trait for the sibling pair, dependent on the number of alleles shared by the siblings. The NP method renders a Z score as test statistic and simply tests for the presence of a QTL. This method makes no assumptions regarding the distribution of the quantitative trait, making it more robust (21). All analyses were performed under the assumption of no dominance variance and all independent sibling pairs within a pedigree were used, with a weighting factor of 2/N for a sibship with N sibs.

Results

Characteristics of the 118 probands and their 257 corresponding siblings are shown in Table 1. The total number of sibling pairs was 554, divided over 58 pedigrees consisting of two siblings and 31 pedigrees with three siblings. The 30 pedigrees that remained contained four siblings or more. Three or more joint sites were affected in 44 percent of probands and 18 percent of siblings, 56 and 49 percent of respectively the probands and the siblings had a combination of

Table 1
Characteristics of the study population.

	Probands (n = 118)	Siblings (n = 257)
Number of Men (%)	33 (28)	124 (48)*
Mean age (SD)	60.9 (2.7)	65.3 (8.0)*
Number with hand ROA (%)	97 (82)	192 (75)
Number with knee ROA (%)	70 (59)	47 (18)*
Number with hip ROA (%)	30 (25)	17 (7)*
Number with DD (%)	95 (81)	213 (83)
Median of sum score (range)†	4.0 (2,9)	4.0 (1,10)
Number with Heberden's nodes (%)	51 (43)	121 (47)

ROA = Radiological osteoarthritis. DD = disk degeneration of the spine.
* Statistically significantly different in comparison with probands ($p < 0.05$).
† Sum score of ROA and disk degeneration of the spine.

two joint sites affected. Finally, 33 percent of the siblings had no or just one joint site affected with ROA or disk degeneration.

COL2A1 gene

For the sum score of ROA and disk degeneration, the variance between siblings in a sibling pair was lower when they shared one or two alleles of the COL2A1 VNTR polymorphism as compared to siblings that shared no alleles (Table 2). This effect was strongest in female pairs, whereas in male pairs almost no effect was observed. For the score summing the number of joints affected with ROA in the hands, the variance was three times lower in female sibling pairs sharing two alleles as compared to female sibling pairs sharing no alleles (LOD = 1.0) (Table 2). For disk degeneration of the spine, analyzed separately, no evidence for linkage with the COL2A1 locus was found (data not shown). When considering the pedigrees of probands with Heberden's nodes, a reduction in variance with an increase in number of alleles shared was observed for the sum score of ROA and disk degeneration and for the sum score of hand ROA. These variances were respectively $\sigma^2_0 = 21.6$, $\sigma^2_1 = 9.9$, and $\sigma^2_2 = 9.9$, with LOD score 0.6 and $\sigma^2_0 = 17.9$, $\sigma^2_1 = 7.6$, and $\sigma^2_2 = 7.6$, with LOD score 0.7. Earlier we reported an association between allele 13R1 (frequency 0.42) of the COL2A1 VNTR polymorphism and ROA in the peripheral joints, i.e. the knees, hips and hands. In an analysis using only the sibling pairs of probands carrying a 13R1 allele with their

Table 2
Quantitative trait locus (QTL) analysis in 554 sibling pairs for the COL2A1 gene.

	Maximum likelihood analysis				Nonparametric analysis	
	σ^2_0	σ^2_1	σ^2_2	LOD score	Z score	LOD score
Sum score of ROA and DD						
All pairs	16.3	12.5	12.5	0.2	-0.2	0.0
Female pairs	18.0	13.3	8.2	0.5	1.3	0.3
Male pairs	14.5	13.5	13.5	0.0	-1.5	0.0
Sum score of hand ROA						
All pairs	12.5	8.6	8.6	0.3	0.1	0.2
Female pairs	14.3	9.8	4.1	1.0	1.6	0.6
Male pairs	10.3	9.3	9.3	0.0	-1.1	0.0

ROA = Radiological osteoarthritis. DD = Disk degeneration. σ^2_0, σ^2_1, σ^2_2 = The variance of the QTL in sibling pairs sharing respectively zero, one or two alleles. Z score = The ratio of the test statistic $X_w(s)$ and square root of the variance V.

respective siblings, a marked decrease in the variance for the sum score of ROA and disk degeneration was observed when siblings shared alleles (σ^2_0 = 16.2, σ^2_1 = 10.0, and σ^2_2 = 6.7, with LOD score 0.2).

COL9A1 gene

For the COL9A1 gene, the variance was equal in all analyses for sibling pairs sharing zero alleles or one allele of the two marker loci STRP-8B2 and 12B1 (Table 3). Sibling pairs sharing two alleles had lower variances as compared with pairs sharing zero or one allele. However, the reduction in variance was minimal.

Discussion

Earlier, we performed population-based association studies to examine the role of the COL2A1 and COL9A1 loci in the occurrence of ROA. The present sibling pair study was performed with probands derived from these population-based studies and their corresponding siblings. For the COL2A1 locus, we earlier observed an association in women between the most frequent allele of the COL2A1

Table 3
Quantitative trait locus (QTL) analysis in 554 sibling pairs for the COL9A1 gene.*

	Maximum likelihood analysis				Nonparametric analysis	
	σ^2_0	σ^2_1	σ^2_2	LOD score	Z score	LOD score
Sum score of ROA and DD						
All pairs	13.8	13.8	13.1	0.1	-0.4	0.0
Female pairs	13.2	13.2	11.2	0.0	-0.1	0.0
Male pairs	15.7	15.7	10.8	0.1	0.1	0.0
Sum score of hand ROA						
All pairs	9.6	9.6	9.6	0.0	-1.2	0.0
Female pairs	9.1	9.1	9.1	0.0	-0.7	0.0
Male pairs	11.1	11.1	6.8	0.2	-0.1	0.1

ROA = Radiological osteoarthritis. DD = Disk degeneration. σ^2_0, σ^2_1, σ^2_2 = The variance of the QTL in sibling pairs sharing respectively zero, one or two alleles. Z score = The ratio of the test statistic $X_w(s)$ and square root of the variance V.
* Results shown are derived from multipoint analyses using marker loci STRPs 12B1 and 8B2 for the COL9A1 gene.

VNTR polymorphism, allele 13R1 (frequency 0.42), and ROA (Chapter 4.1). The findings on the COL2A1 locus in the present study, which show a reduction in variance for OA as quantitative trait with an increase in the number of alleles shared between female siblings, are in line with the detected association between the COL2A1 locus and ROA. For the COL9A1 locus, an association was earlier observed between STRP-12B1 (a marker locus for the COL9A1 gene) and ROA that was stronger in women as compared to men (Chapter 4.2). In the present study, no evidence for linkage was found between the sum score of ROA and disk degeneration, hand ROA, or disk degeneration of the spine and the COL9A1 locus.

The most important limitation of our study concerns the statistical power. Although, 554 sibling pairs were available for analyses, no significant results could be reported. The maximum LOD score that was reached amounted to 1.0 with 80 percent ability to extract the full IBD status in case of the COL9A1 locus, where two closely linked marker loci were used. In case of the COL2A1 VNTR polymorphism full IBD status could be calculated for 68 percent of all sibling pairs.

Previously, one sibling pair study of limited size concerning the COL2A1 locus has been published (10). This affected sibling pair study did not show evidence for linkage of OA in three or more joint sites before the age of 60 years to the COL2A1 locus. The strongest indications for linkage in the present sibling pair study were observed in two out of three subgroups that earlier showed the strongest association between this locus and ROA, i.e. women, in particular those with Heberden' s nodes. These findings further support the hypothesis that the COL2A1 locus plays a role in the development of ROA in peripheral joints. However, given the limited size of the present study these findings are not conclusive.

In case of the COL9A1 locus no earlier association or sibling pair study has been performed. In our previous association study, we found no allele at the COL9A1 locus predisposing for the occurrence of ROA or disk degeneration, but instead observed an association indicating a possibly protective effect of this locus. The present sibling pair study mainly consisted of siblings that were affected with ROA and/or disk degeneration (only 12 siblings were free from ROA and disk degeneration). Therefore, it was unlikely that in this sample it would be possible to confirm this protective effect, because of a lack of statistical power. Alternatively, the present study supports the finding of our previous association study that the COL9A1 locus does not have a predisposing effect on the occurrence of ROA or disk degeneration.

In conclusion, findings of the present study are compatible with a role of the COL2A1 locus in the occurrence of ROA in peripheral joints, although in the current setting no statistically significant results were observed. We could not

find evidence that the COL9A1 locus increases the risk of developing ROA or disk degeneration.

References

1. Spector TD, Cicuttini F, Baker J, Loughlin J, Hart D. 1996. Genetic influences on osteoarthritis in women: a twin study. BMJ 312:940-3.
2. Felson DT, Couropmitree NN, Chaisson CE, Hannan MT, Zhang Y, McAlindon TE, LaValley M, Levy D, Myers RH. 1998. Evidence for a mendelian gene in a segregation analysis of generalized radiographic osteoarthritis. Arthritis Rheum 41:1064-71.
3. Hirsch R, Lethbridge-Cejku M, Hanson R, Scott WW, Reichle R, Plato CC, Tobin JD, Hochberg MC. 1998. Familial aggregation of osteoarthritis. Arthritis Rheum 41:1227-32.
4. Hull R and Pope FM. 1989. Osteoarthritis and cartilage collagen genes. Lancet i:1337-8.
5. Loughlin J, Irven C, Athanasou N, Carr A, Sykes B. 1995. Differential allelic expression of the type II collagen gene (COL2A1) in osteoarthritic cartilage. Am J Hum Genet 56:1186-93.
6. Loughlin J, Irven C, Athanasou N, et al. Differential allelic expression of the type II collagen gene (COL2A1) in osteoarthritic cartilage. Am J Hum Genet 1995;56:1186-93.
7. Meulenbelt I, Bijkerk C, Miedema HS, Breedveld FC, Hofman A, Valkenburg HA, Pols HAP, Slagboom PE, van Duijn CM. A genetic association study of the IGF-1 gene and radiological osteoarthritis in a population-based cohort study (the Rotterdam Study). Ann Rheum Dis 1998;57:371-4.
8. Meulenbelt I, Bijkerk C, de Wildt SC, Miedema HS, Valkenburg HA, Breedveld FC, Pols HAP, te Koppele JM, Sloos VF, Hofman A, Slagboom PE, van Duijn CM. Investigation of the association of the CRTM and CRTL1 genes with radiographically evident osteoarthritis in subjects from the Rotterdam Study. Arthritis Rheum 1997;40:1760-5.
9. Vikkula M, Nissilä M, Hirvensalo E, Nuotio P, Palotie A, Aho K, Peltonen L. 1993. Multiallelic polymorphism of the cartilage collagen gene: no association with osteoarthritis. Ann Rheum Dis 52:762-4.
10. Loughlin J, Irven C, Fergusson C, Sykes B. 1994. Sibling pair analysis shows no linkage of generalized osteoarthritis to the loci encoding type II collagen, cartilage link protein, or cartilage matrix protein. Br J Rheumatol 33:1103-6.
11. Aerssens J, Dequeker J, Peeters J, Breemans S, Boonen S. 1998. Lack of association between osteoarthritis of the hip and gene polymorphisms of VDR, COL1A1, COL2A1 in postmenopausal women. Arthritis Rheum 41:1946-50.
12. Diab M, Wu JJ, Eyre DR. 1996. Collagen type IX from human cartilage: a structural profile of intermolecular cross-linking sites. Biochem J 314:327-32.
13. Weeks DE, Lathrop GM. 1995. Polygenic disease: methods for mapping complex disease traits. Trends Genet 11:513-9.
14. Hofman A, Grobbee DE, De Jong PVTM, van den Ouweland FA. 1991. Determinants of disease and disability in the elderly. Eur J Epidemiol 7:403-422.
15. Silman AJ, Hochberg MC 1993. The epidemiology of the rheumatic diseases. Oxford: Oxford University Press 257-88.
16. Kimura T, Nakata K, Tsumaki N, Miyamoto S, Matsui Y, Ebara S, Ochi T 1996. Progressive degeneration of articular cartilage and intervertebral discs. An experimental study in transgenic mice bearing a type IX collagen mutation. Int Orthopaed 20:177-81.

17. Kellgren JH, Jeffrey MR, Ball J (editors). 1963. The epidemiology of chronic rheumatism. Volume II: Atlas of standard radiographs of arthritis. Oxford: Blackwell Scientific Publications.
18. Berg ES, Olaisen B. 1993. Characterization of the COL2A1 VNTR polymorphism. Genomics 16:350-4.
19. Warman ML, Tiller GE, Polumbo PA, Seldin MF, Rochelle JM, Knoll JHM, Cheng SD, Olsen BR. 1993. Physical and linkage mapping of the human and murine genes for the α1 chain of type IX collagen (COL9A1). Genomics 17:694-8.
20. Kruglyak L, Lander E. 1995. Complete multipoint sib pair analysis of qualitative and quantitative traits. Am J Hum Genet 57:439-54.
21. Kruglyak L, Lander E. 1995. A nonparametric approach for mapping quantitative trait loci. Genetics 139:1421-8.

5

Gene Interaction in Osteoarthritis

The IGF-1 gene and radiological osteoarthritis in a population-based study

Abstract

Objective *A genetic association study was performed to investigate whether radiological osteoarthritis (ROA) was associated with specific genotypes of the insulin-like growth factor I (IGF-1) gene.*

Methods *Subjects aged 55-65 years were selected from a population-based study of which ROA at the knee, hip, and hand and disk degeneration of the spine was assessed. Genotypes were determined of a polymorphism in the promoter region of the IGF-1 gene.*

Results *The IGF-1 locus was significantly associated with the presence of ROA (overall adjusted OR for heterozygous subjects = 1.9, 95% CI 1.2-3.1 and for homozygous subjects 3.6, 95% CI 0.8-16.2).*

Conclusion *Our results suggest that variation at the IGF-1 locus is associated with ROA and/or disk degeneration and may play a role in OA pathogenesis. To confirm these findings replication in another population based sample is needed.*

Introduction

Osteoarthritis (OA) is a disease characterized by the degradation of articular cartilage and formation of new bone (osteophytes and sclerosis). Several reports suggest that genetic influences contribute considerably to the development of OA (1,2). The relevance of the genetic component, however, varies among subgroups of patients and as yet it is not clear which genes are involved (3).

Insulin-like growth factor 1 (IGF-1) stimulates chondrocytes to synthesize extracellular matrix (ECM) components in cartilage (4,5). Its action is mediated through the type 1 IGF receptor. The function of IGF-1 and its receptor in cartilage formation both during developmental stages and remodeling of adult cartilage may be relevant to the etiology of OA. IGF-1 may also influence OA by osteophyte formation (6). Although osteophyte growth during OA progression and bone mineral density (BMD) is positively correlated to high serum IGF-1 levels (6,7), no consistent relationship between serum IGF-1 levels and OA has been found (8,9). Furthermore, serum IGF-1 is inversely correlated to age (10) and body mass index (BMI) (11). Since individual IGF-1 levels are liable to temporal variations, assessing the role of IGF-1 in OA by serum levels is complex. We have examined the relation between the IGF-1 gene and the presence of radiological OA (ROA) in subjects aged 55-65 years.

Materials and methods

Subjects

Individuals were derived from a prospective population-based cohort study of determinants and prognosis of chronic diseases in the elderly, the Rotterdam study (12). Weight bearing anterior-posterior radiographs of the hips and knees, anterior-posterior radiographs of the hands and wrists and lateral radiographs of the spine (Th4-S1) were obtained from a random population of 944 unrelated individuals aged 55-65 years. ROA and disk degeneration was assessed by two independent readers and by means of the grading system proposed by Kellgren (13). By applying these criteria a hip joint with Kellgren score 2 requires the presence of both definite joint space narrowing and definite osteophytes. A knee or hand joint with Kellgren score 2 requires the presence of definite osteophytes and possible narrowing of joint space. A spine joint with Kellgren score 2 denotes definite lateral osteophytes. For both knees and hips radiographs had previously been scored (14). ROA of the hand was assessed in each inter- and metacarpalphalangeal joint individually, and the first carpometacarpal and trapezoscaphoideal joint. ROA of the wrist was assessed at the radiocarpal and

distal radioulnar joints. For the spine three levels, from Th4 to S1, were scored with regard to osteophytes and disk space narrowing, e.g. thoracic, lumbar and lumbosacral. In the analysis definite ROA was defined as Kellgren-score 2 or over in the left and/or right corresponding joint. Hand ROA was defined as Kellgren-score 2 or over in at least one of the 36 joints that were scored. For this purpose the joints of the wrist were included in the category hand ROA. Disk degeneration of the spine was defined as Kellgren-score 2 or over in at least one out of three levels scored. The presence or absence of osteophytes of the knee was scored separately.

Information on age (in years), BMI (measured as weight in kg divided by height^2 in metres), and BMD (measured as gram mineral divided by area in cm^2) of the neck of the femur were used in the study. In view of the hypothesis that the genetic contribution to ROA differs in men and women and may depend on the joint site, stratified analysis was performed with respect to gender and joint site.

Genotyping and Statistical analysis

Genotypes of the dinucleotide repeat polymorphism of the IGF-1 gene were determined as previously described (15) of 786 individuals for whom cells were available. Demographic variables (sex, age, BMI and BMD) were compared between subjects with and without ROA by using t-tests for independent samples. Counting alleles and calculating sample proportions assessed allele frequencies. Chi square (X^2) for Hardy Weinberg equilibrium (HWE) were calculated using the HWE-program (LINKUTIL package) (16). Alleles with an allele frequency < 0.05 were pooled. A likelihood ratio test was used to test for association of IGF-1 alleles with the occurrence of ROA (17). This method is specifically suitable to perform association studies with polymorphic markers with multiple alleles. To measure the strength of association between ROA and IGF-1 genotypes, a logistic regression model was used to estimate the odds ratio (OR). ORs were adjusted for risk factors of OA i.e., sex, age, BMI and BMD. ORs are presented with 95% confidence intervals (95% CI). The statistical package SPSS was used and P-values < 0.05 (2-sided) were considered significant.

Results

Table 1 shows the number of genotyped individuals with ROA and/or disk degeneration of the spine (ROA+) and with none of the four joint sites affected (ROA-) and the mean age, body mass index (BMI), and bone mineral density (BMD). The mean age and BMI differed significantly between individuals with and without ROA and/or disk degeneration ($p < 0.001$). Only 17% of individuals

were free of ROA and disk degeneration of the spine in every joint investigated in this relatively young age group.

In total 9 different alleles were identified (A1-A9) with allele frequencies ranging from 0.002-0.66. IGF-1 allele frequencies of the 4 most frequent alleles are shown in Table 1. Except for the relatively rare allele A2, the IGF-1 allele frequencies observed in the random population, and the subjects with and without ROA and/or disk degeneration were similar as described by Weber and May (15) (see Table 1). The low frequency alleles A1 (frequency 0.02), A6 (frequency 0.02), A7 (frequency 0.003), and A9 (frequency 0.002) (not shown in Table 1) were not previously described in a population-based study (15). The distribution of genotype frequencies was not significantly different from that expected for a population in HWE neither overall (P = 0.76) nor for the ROA+ (p = 0.78) or the ROA- group (p = 0.47).

Table 1 shows association of the IGF-1 polymorphism in ROA+ as compared to ROA- subjects (p = 0.02). Differences in allele frequency A3 and A4 between ROA+ and ROA- subjects caused this association. The strength of the association of the IGF-1 locus with ROA was estimated using a logistic regres-

Table 1
Characteristics of random sample of 786 individuals aged 55 to 65 years with allele frequencies of the dinucleotide repeat polymorphism in the promotor region of the IGF-1 gene.

	Overall	ROA+	ROA-
Total number of individuals	786	651	135
Characteristics of subjects			
Number of men (%)	317 (40)	254 (39)	63 (47)
Age in years (SD)		60.5 (2.7)*	59.5 (2.7)
BMI in kg/m^2 (SD)		26.6 (3.7)*	25.1 (3.1)
BMD in g/cm^2 (SD)		0.87 (0.13)	0.86 (0.12)

Alleles**	Allele frequencies (number of alleles)				LRT‡	p-value
	Weber† (88)	Overall (1572)	ROA+ (1302)	ROA- (270)		
A2	0.18	0.07	0.07	0.07	4.9	0.02
A3	0.16	0.19	0.20	0.13		
A4	0.60	0.66	0.65	0.72		
A5	0.06	0.05	0.05	0.05		
other	0.00	0.04	0.04	0.04		

* significantly different from the control group with $p < 0.01$
** IGF-1 alleles with nomenclature as in Weber and May (15)
† allele frequencies as in Weber and May (15)
‡ likelihood-ratio test statistic = $-2\ln[L(H_0)/L(H_1)]$

sion model (Table 2). The overall adjusted OR (age, sex, BMI, and BMD) for ROA+ subjects heterozygous for IGF-1 allele A3 was 1.9, 95% CI 1.2-3.1. For subjects homozygous for IGF-1 allele A3 (A3/A3) the overall adjusted OR (age, sex, BMI, and BMD) was 3.6, 95% CI 0.8-16.2. A protective effect was observed for the IGF-1 allele A4 with an overall adjusted OR (age, sex, BMI, and BMD) for heterozygous ROA+ subjects of 0.7, 95% CI 0.4-1.5 and an overall adjusted OR of 0.5, 95% CI 0.3-1.0 for homozygous A4/A4 subjects. Interaction in these analyses with sex, age, BMI, and BMD was not observed.

Since IGF-1 allele A3 shows a significant OR and is rarer than allele A4 it is, from a population genetic point, the most likely allele associated to ROA. We, therefore, have chosen to further investigate the effect of allele A3. To retain sufficient numbers (power) the homo- and heterozygous genotypes with allele A3 were added and used to perform stratified analysis by gender and by sepa-

Table 2
Odds ratios of subjects with IGF-1 genotypes containing allele 3 as compared to all other genotypes.

Subjects	Number with A3 genotype (frequency)				
	-/-	-/+	OR (95% CI)*	+/+	OR (95% CI)*
ROA-	103 (0.76)	30 (0.22)	Reference	2 (0.01)	Reference
ROA+	417 (0.64)	211 (0.32)	1.9 (1.2-3.1)	23 (0.04)	3.6 (0.8-16.2)

* Odds ratio adjusted for age, body mass index, bone mineral density.

Table 3
Odds ratios of subjects with IGF-1 genotype containing allele 3 (homo and heterozygote) as compared to all other genotypes.

Subjects	N	number with other genotypes (frequency)	number with A3 genotypes (frequency)	crude OR (95% CI)	adjusted OR (95% CI)*
total	786	520 (0.66)	266 (0.34)		
ROA-	135	103 (0.76)	32 (0.24)	Reference	Reference
ROA+	651	417 (0.64)	234 (0.36)	1.8 (1.2-2.8)	2.0 (1.3-3.1)
Knee ROA	142	96 (0.68)	46 (0.32)	1.5 (0.9-2.6)	1.8 (1.0-3.4)
Hip ROA	71	42 (0.59)	29 (0.41)	2.3 (1.2-4.2)	2.8 (1.4-5.7)
Hand ROA	444	284 (0.64)	160 (0.36)	1.8 (1.2-2.9)	2.2 (1.4-3.5)
DD Spine	479	308 (0.64)	171 (0.36)	1.8 (1.2-2.9)	2.0 (1.2-3.2)

* Odds ratio adjusted for age, body mass index, bone mineral density.
DD = disk degeneration

rate joint sites in ROA+ subjects. The overall adjusted OR calculated for homo- and heterozygous carriers of the A3 allele together using the remaining genotypes as reference was 2.0, 95% CI 1.3-3.1. This association with IGF-1 A3 genotypes was found in both men (adjusted OR = 2.4, 95% CI 1.2-4.8) and women (adjusted OR = 1.8, 95% CI 1.0-3.3) being strongest in men. When subjects were selected on joint site specific ROA, a significant effect of the IGF-1 genotype with allele A3 was observed for each individual joint (Table 3). The strongest effect as measured by the OR was observed in subjects with hip ROA with an adjusted OR of 2.8, 95% CI 1.4-5.7. When subjects were stratified by number of joint groups affected (knee, hip, hand, and spine) the strength of the association measured by the OR of A3 genotypes was not higher for individuals with ROA in 3 joint sites (generalized ROA).

Discussion

We investigated whether a polymorphic marker of the IGF-1 gene is associated with the presence of ROA at the knee, hip, or hand or with disk degeneration of the spine. In a population-based study of 786 subjects, the frequency of the subjects heterozygous for the IGF-1 allele A3 was found to be approximately two times increased in ROA+ subjects as compared to ROA- subjects (adjusted OR 1.9, 95% CI 1.2-3.1). A 3.5 times increased frequency of the A3/A3 homozygous IGF-1 genotype was observed among subjects with ROA+ (adjusted OR 3.6, 95% CI 0.8-16.2). The latter association was not significant, most likely because of the small number of subjects homozygous for IGF-1 allele A3 (for ROA+ n=23, for ROA- n= 3). The associations observed were not explained by age, sex, BMD, or BMI (7,10,11). The deviation of IGF-1 allele 4 may be a consequence of compensating allele frequencies. Moreover, no association was found between IGF-1 alleles and BMD or BMI nor did we observe an association of IGF-1 locus with the presence of osteophytes in the knee as has been suggested for serum IGF-1 levels (6) (results not shown). Since association studies are subject to false positive results the observed associations require cautious interpretation and replication in a second population based study.

In view of the function of IGF-1 in articular cartilage metabolism and the intragenic location of the dinucleotide repeat polymorphism, the association found may be due to a role of the IGF-1 gene in the onset of ROA. We, however, cannot exclude the possibility that a gene closely linked to the IGF-1 locus influences the association. Two other genes on chromosome 12 which may play a role in the onset of OA are the procollagen type II (COL2A1) (18) and the vitamin D receptor (VDR) genes (19). The location of these genes, however, is on 12q12-14, which is at least 80 cM of the IGF-1 gene. Since in unrelated subjects

linkage disequilibrium extends only over short genetic distances (1-2 cM), it is not likely that mutations in these genes cause the currently described associations.

In addition to IGF-1, genotypes of a polymorphism in the gene encoding the receptor of the IGF-1 gene (20) were studied but did not show any association in subjects with ROA in the knee and/or the hip as compared to subjects without ROA (results not shown).

Although the IGF-1 polymorphism is located in the promotor region, and may affect the expression of the gene, its relation to IGF-1 levels is not yet known. The association observed may be explained by differences in carriers versus non-carriers in the response of the chondrocyte, via IGF-1, to cartilage damage and degradation during the OA process (4,5). The observation that IGF-1 allele A3 is associated with ROA at any joint site (knee, hip, hand, and spine) and does not increase specifically for subjects with generalized ROA may indicate a mild genetic predisposing effect, which phenotypic outcome may depend on other factors e.g. mechanical stress. Carriers of the IGF-1 allele A3 may thus be predisposed to ROA at any possible joint.

The observed association was strongest in subjects with hip ROA, a site for which the role of genetic factors was not previously assessed. It has, however, been reported that ROA of the hip has a specific sex and geographically prevalence pattern, which may suggest the involvement of systemic factors to the onset of hip ROA. Furthermore, hip ROA is often considered to arise due to an anatomical abnormality (21). Since IGF-1 is expressed during developmental stages and plays an important role in cartilage formation (22), the effect of the IGF-1 locus may be exerted via this way.

In our population-based study we were able to study specifically the pathophysiology of ROA of the hip, knee, hand, and spine. Our selection criteria for ROA negative subjects were strict in that we included only subjects without ROA at any of the 4 joints studied. Due to the high prevalence of hand and spine ROA only 135 (17%) ROA negative subjects fulfilled these criteria. This may have contributed to the detection of association since the observed association was mainly due to reduced frequency of allele A3 in subjects without ROA, suggesting that especially absence of this allele in the genotype lowers the risk for ROA. It is also possible that another IGF-1 allele protects subjects without ROA. The frequency of IGF-1 allele 4 in this respect is higher among subjects without ROA and may possibly protect. The relative high allele frequency of this allele, however, may have decreased power to significantly prove such a protective effect. The independent effect of allele A3 and A4 could not be tested by exclusion of individuals with either IGF-1 allele since the remaining number of individuals was not sufficient.

Our study shows an association of IGF-1 genotype with the prevalence of ROA in knee, hip, hand, or disk degeneration of the spine irrespective of BMD, BMI, and age. These findings suggest that IGF-1 plays a role in OA pathogenesis.

References

1. Spector TD, Cicuttini F, Baker J, Loughlin J, Hart DJ. Genetic influences on osteoarthritis in women: a twins study. BMJ 1996;312:940-4.
2. Kellgren JH, Lawrence S, Bier F: Genetic factors in generalized osteoarthrosis. Ann Rheum Dis 1963;22:237-253.
3. Cicuttine FM and Spector TD What is the evidence that osteoarthritis is genetically determined? Baill Clin Rheum 1997;11:657-669.
4. McQuillan DJ, Handley CJ, Campbell MA, Bolis S, Milway VE, Herington AC. Stimulation of proteoglycan biosynthesis by serum and insulin-like growth factor-1 in cultured bovine articular cartilage. Biochem J 1986;240:423-430.
5. Schoenle E, Zapf J, Humber RE, Froesch ER. Insulin-like growth factor I stimulates growth in hypophysectomized rats. Nature 1982;296:252-253.
6. Schouten JSAG, Van den Ouweland FA, Valkenburg HA, Lamberts SWJ. Insulin-like growth factor-1: a prognostic factor of knee osteoarthritis. Br J Rheumatol 1993;32:274-280.
7. Dequeker J, Mohan S, Finkelman D, Aerssens J, and Baylink DJ (1993) Generalized osteoarthritis associated with increased insulin-like growth factor types II and transforming growth factor ß in cortical bone from thee iliac crest. Arthritis and Rheumatism 1993;36:1702-8.
8. Lloyd ME, Hart DJ, Nandra D, McAlindon TE, Wheeler M, Doyle DV, Spector TD. Relation between insulin-like growth factor-I concentrations, osteoarthritis, bone density, and fractures in the general population: the Chingford study. Ann Rheum Dis 1996;55:870-4.
9. Denko CW, Boja B, Moskowitz RW. Growth promoting peptides in osteoarthritis: insulin, insulin-like growth factor-1, growth hormone. J Rheumatol 1990;17:1217-21.
10. Bennet AE, Wahner HW, Riggs BL, Hintz RL. Insulin-like growth factors I and II: Aging and bone density in women. J Clin Endocrinol Metab 1984;59:701-4.
11. Rudman D, Feller AG, Hoskote SN, Gergans GA, Lalitha PY, Goldberg AF. Effects of growth hormone in men aged over 60 years old. N Engl J Med 1990;323:1-6.
12. Hofman A, Grobbee DE, De Jong PTVW, Van den Ouweland FA: Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidem 1991;7:403-22.
13. Kellgren JH, Lawrence JS. Atlas of Standard Radiographs; The Epidemiology of Chronic Rheumatism. Vol 2 Oxford: Blackwell Scientific Publications, 1963.
14. Odding E, Valkenburg HA, Algra D, van den Ouweland FA, Grobbee DE, Hofman A. Associations of radiological osteoarthritis of the hip and knee with locomotor disability in the Rotterdam Study. Ann Rheum Dis 1998;57:203-8.
15. Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 1989;44:388-96.
16. Ott J. Analysis of human genetic linkage, revised ed. Baltimore: John Hopkins University Press, 1991
17. Terwilliger JD. A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic marker loci. Am J Hum Genet 1995;56:777-87.

18. Williams CJ, Jimenez SA. Heredity, genes and osteoarthritis. Rheum Dis Clin North Am 1993; 19:523-43.
19. Keen RW, Hart DJ, Lanchbury, and Spector TD. Association of early osteoarthritis of the knee with a Taq I polymorphism of the vitamin D receptor gene. Arthritis Rheum 1997;40:1444-9.
20. Meloni R, Fougerousse F, Roudaut C, Beckmann JS. Trinucleotide repeat polymorphism at the human insulin-like growth factor I receptor gene (IGF-1R). Nucleic Acids Res 1992;20:1427.
21. Harris W. Etiology of osteoarthritis of the hip. Clin Orthop 1986;213:20-33.
22. Humbel RE. Insulin-like growth factors I and II. Eur J Biochem 1990;190:445-62.

Interaction between the IGF-1 and COL2A1 genes in the association with radiological osteoarthritis

Abstract

Objective *We performed a study to investigate a possible interaction between the IGF-1 and COL2A1 genes on the occurrence of radiological osteoarthritis (ROA) and of disk degeneration of the spine.*

Methods *In 783 subjects, aged 55 to 65 years, derived from a population-based study ROA of the knees, hips and hands and disk degeneration of the spine was assessed. Genotypes were determined of a polymorphism in the promotor region of the IGF-1 gene and a VNTR polymorphism located 1.35 kb to the 3' end of the COL2A1 gene.*

Results *The prevalence of ROA and/or disk degeneration was significantly increased in individuals who carry both the A3 allele of the IGF-1 gene and the 13R1 allele of the COL2A1 gene as compared to individuals carrying none of these alleles (odds ratio (OR) = 2.2, 95% CI 1.1-4.3). Stratification according to affected joint site showed for all four joint sites a similar interactive effect. The effects were strongest for individuals homozygous for allele 13R1 (OR = 4.7, 95% CI 1.0-21.0) and for individuals with Heberden's nodes (OR = 4.0, 95% CI 0.7-22.5). There was no evidence for an effect of COL2A1 allele 13R1 in the absence of the IGF-1 allele A3 or for an effect of the IGF-1 allele A3 in the absence of COL2A1 allele 13R1.*

Conclusion *The present study suggests that the IGF-1 and COL2A1 genes are involved in the occurrence of ROA and/or disk degeneration of the spine through a mechanism in which both genes interact.*

Introduction

Radiological osteoarthritis (ROA) of the peripheral joints is to a large extent determined by genetic factors (1-3). The influence of the genetic component varies between different subgroups of osteoarthritis (OA) phenotypes, e.g. hand ROA or knee ROA (see Chapter 3.2). Several candidate genes that could play a role in the occurrence of OA have been investigated, of which some have shown an association with common forms of OA (4). However, interaction between genes, through which the genetic influence on OA is probably exerted, has not been investigated.

Previously, we have reported an association between a polymorphism in the promotor region of the insulin-like growth factor 1 (IGF-1) gene and ROA in a population-based study (5). This polymorphism is associated with serum IGF-1 level (6). IGF-1 mediates the effects of growth hormone at the local tissue level and is important for proteoglycan synthesis by chondrocytes and for bone remodeling, i.e. bone cell proliferation, differentiation, and collagen synthesis (7-9). The relationship between serum IGF-1 levels and OA is unclear, since high, normal and low levels of IGF-1 have been reported in individuals with OA (10-13).

Several mutations in the COL2A1 gene, encoding the predominant cartilage collagen type II, have been reported to lead to severe early-onset OA often with chondrodysplasia (14). Findings on the role of the COL2A1 gene in the occurrence of common forms of OA, with onset later in life, remain controversial. Previously, we found an association between the most common allele of the COL2A1 VNTR polymorphism, the 13R1 allele (frequency 0.42), and ROA in women (see Chapter 4.1).

The aim of the present study was to test for interaction between the IGF-1 gene and the COL2A1 gene on the occurrence of ROA in the knees, hips, and hands and of disk degeneration of the spine.

Methods

Subjects

All individuals were derived from a prospective population-based cohort study of determinants and prognosis of chronic diseases in the elderly (15). In order to

ascertain ROA of relatively early-onset and herewith to distinguish genetic predisposition from environmental determinants of OA, the study was restricted to non-institutionalized individuals aged 55 to 65 years. Weight-bearing radiographs of the hips and knees, anteroposterior radiographs of the hands and lateral radiographs of the spine were obtained in a random sample of 944 individuals. ROA was assessed by means of the Kellgren grading system (16) in five grades (0-4). Two independent readers, who were blinded to all other data of the participant, scored all radiographs. Radiographs of the knees and hips had previously been scored (17). ROA of the hand was assessed in each inter- and metacarpalphalangeal joint, the first carpometacarpal, the trapezoscaphoideal, the radionavicular and the distal radioulnar joints. Definite knee or hip ROA was defined as a Kellgren-score two or over in the left and/or right corresponding joint. Definite hand ROA was defined as a Kellgren-score two or over in at least one of the 36 joints that were scored in both hands. In the Kellgren grading system mild ROA (grade 2) of the hip requires both narrowing of joint space and definite osteophytes, whereas mild ROA of the knee or hand only requires definite osteophytes with possible narrowing of joint space.

ROA of the spine could not be assessed on the lateral radiographs of the spine that were available. Instead, disk degeneration of the spine was assessed at three levels, i.e. thoracic (Th4 to Th12), lumbar (L1 to L4) and lumbosacral (L5-S1 or L5-L6). Disk degeneration was scored using the Kellgren grades (0-4), in which a grade two denotes vertebral osteophytosis and grades three and four vertebral osteophytosis accompanied by respectively moderate or severe disk space narrowing.

Body height (in cm) and weight (in kg) was measured under standardized conditions. Body mass index (in kg/m^2) was used as a measure of obesity. Bone mineral density (in g/cm^2) was measured at the femoral neck by dual energy x-ray absorptiometry as described previously (18).

Genotyping and statistical analysis

Genotypes of a VNTR polymorphism located 1.35 kb to the 3' end of the COL2A1 gene and of a dinucleotide polymorphism in the promotor region of the IGF-1 gene were determined as described previously (19, 20). DNA was available and genotyping was successful in 783 individuals (83 percent). In total, 16 alleles of the COL2A1 VNTR polymorphism and 9 alleles of the IGF-1 gene polymorphism were detected. All genotyping was performed without knowledge of any other data of the participant.

Demographic variables were compared using the Student's *t* test and the chi-square test. Allele frequencies were assessed by counting alleles and calculating sample proportions. To measure the strength of the association between ROA and/or disk degeneration and COL2A1- or IGF-1 genotypes, multiple logis-

tic regression analysis was used to estimate the odds ratio (OR). ORs are presented with 95 percent confidence intervals (CI) and were adjusted for age, sex, body mass index, and bone mineral density. In the analyses, individuals with ROA and/or disk degeneration were compared with individuals free from both ROA and disk degeneration.

Results

The baseline characteristics of the study population are shown in Table 1. ROA and/or disk degeneration was present in at least one of four joint sites, i.e. knees, hips, hands, and spine, in 648 individuals (83 percent). Only 133 individuals (17 percent) were free of ROA and disk degeneration at all sites. Relatively more men were free of radiographic abnormalities than women, although this was not statistically significant. Both mean age and body mass index were statistically significantly higher in affected individuals as compared to not affected individuals.

Previously, we found alleles of both loci associated with ROA and/or disk degeneration, i.e. allele A3 of a polymorphism in the promotor region of the IGF-1 gene and allele 13R1 in case of the COL2A1 VNTR polymorphism (see Chapters 4.1 and 5.1). To examine a possible interactive effect between these two loci, individuals carrying either the IGF-1 allele A3 or the COL2A1 allele 13R1 or carrying both risk alleles were compared with individuals without the IGF-1 and COL2A1 risk alleles.

Table 2 shows that the occurrence of ROA and/or disk degeneration is statistically significantly higher in individuals carrying risk alleles at both loci as compared to individuals carrying none of these alleles (OR = 2.2, 95% CI 1.1-4.3). There was no statistically significant evidence for an association between

Table 1
Characteristics study population

	ROA and/or disk degeneration present	ROA and disk degeneration absent
Number of individuals	648	133
Number of men (%)	253 (39)	62 (47)
Age in years (SD)	60.5 (2.7)	59.5 (2.7)*
BMI in kg/m^2 (SD)	26.5 (3.7)	25.1 (3.1)*
BMD in g/cm^2 (SD)	0.87 (0.13)	0.86 (0.12)

** $p < 0.05$ in comparison with individuals with ROA and/or disk degeneration. ROA = radiological osteoarthritis. BMI = body mass index. BMD = bone mineral density.

COL2A1 allele 13R1 and ROA and/or disk degeneration in the absence of IGF-1 allele A3 (OR = 1.0, 95% CI 0.6-1.6). Nor was there evidence for a significant association between IGF-1 allele A3 and ROA and/or disk degeneration in the absence of COL2A1 allele 13R1 (OR = 1.3, 95% CI 0.6-2.6). Stratification according to affected joint site showed for all four joint sites a similar pattern (see Table 2).

Previously, we observed that for the COL2A1 locus the strongest association was present in individuals homozygous for allele 13R1 (see Chapter 4.1). For the interactive effect in combination with the IGF-1 gene, we performed the

Table 2
Interaction between allele A3 of the IGF-1 gene and allele 13R1 of the COL2A1 gene on the occurrence of radiological OA (ROA) and/or disk degeneration.*

		COL2A1 **13R1** - OR (95 % CI)†	COL2A1 **13R1** + OR (95 % CI)†
Overall	IGF-1 **A3** -	Reference	1.0 (0.6-1.6)
	A3 +	1.3 (0.6-2.6)	2.2 (1.1-4.3)‡
Knee ROA	IGF-1 **A3** -	Reference	0.7 (0.3-1.4)
	A3 +	0.5 (0.1-1.4)	1.8 (0.7-4.4)
Hip ROA	IGF-1 **A3** -	Reference	0.8 (0.4-1.9)
	A3 +	1.3 (0.4-4.1)	2.7 (1.0-7.6)‡
Hand ROA	IGF-1 **A3** -	Reference	1.0 (0.6-1.7)
	A3 +	1.1 (0.5-2.3)	2.2 (1.1-4.4)‡
Disk degeneration	IGF-1 **A3** -	Reference	0.9 (0.5-1.5)
	A3 +	1.3 (0.6-2.7)	2.2 (1.1-4.3)‡

* Next to an overall analysis, individual joint sites were investigated.
† All ORs are adjusted for age, sex, body mass index and bone mineral density.
‡ $p < 0.05$

Table 3
Interaction between IGF-1 allele A3 and COL2A1 allele 13R1, stratified according to hetero- or homozygosity for allele 13R1 of the COL2A1 gene. Presented are odds ratios (OR) with 95% confidence intervals (CI).

	COL2A1 genotypes		
	13R1-/ 13R1-	**13R1+/13R1-**	**13R1+/13R1+**
IGF-1 genotypes			
A3-	Reference	1.0 (0.6-1.6)	1.0 (0.5-2.1)
A3+	1.3 (0.6-2.6)	2.0 (1.0-3.9)*	4.7 (1.0-21.0)*

All ORs are adjusted for age, sex, body mass index and bone mineral density.
* $p < 0.05$

analyses for COL2A1 allele 13R1 heterozygotes and homozygotes separately. Table 3 shows that the interactive effect of both genes was strongest in individuals homozygous for allele 13R1 (OR = 4.7, 95% CI 1.0-22.0). Also, individuals with Heberden's nodes showed a stronger association between ROA and/or disk degeneration and carriership of both risk alleles than individuals without Heberden's nodes (Heberden's (+) OR = 4.0, 95% CI 0.7-22.5 versus Heberden's (-) OR = 1.7, 95% CI 0.8-3.5).

Discussion

Earlier we found an association between a frequent allele of a polymorphism in the promotor region of the IGF-1 gene and ROA and/or disk degeneration (5). For the COL2A1 gene, another major candidate gene for OA, we found evidence for a role of the most frequent allele of a VNTR polymorphism in the occurrence of ROA (Chapter 4.1). In the present study we observed that the association of allele 13R1 of the COL2A1 gene with ROA and/or disk degeneration was only present in carriers of allele A3 of the IGF-1 gene, suggesting an interactive effect between both loci. Also, the effect of the IGF-1 A3 risk allele was conditional on the presence of the COL2A1 risk allele 13R1. The interaction was a generalized effect that was not observed joint site specific.

The observed interactive effect between IGF-1 allele A3 and COL2A1 allele 13R1 was independent of the effects of age, sex, body mass index, and bone mineral density. The Vitamin D receptor (VDR) gene is located at a distance of 750 kb from the COL2A1 gene and was found to be associated with knee ROA and bone mineral density (21, 22). We can not exclude the possibility that the VDR gene is contributing to the observed interaction between the IGF-1 and COL2A1 loci. However, the fact that this interactive effect was independent of bone mineral density suggests that a role of the VDR gene is unlikely. When testing for interaction, the interaction term was not statistically significant. However, the statistical power of this analysis was low. We did not investigate for interactive effects between other alleles of both loci, because in our earlier association studies no other alleles were statistically significantly associated with the occurrence of ROA and/or disk degeneration.

In a recent study the currently investigated polymorphism in the promotor region of the IGF-1 gene was associated with the serum IGF-1 level (6). Carriers of allele A3 of this polymorphism had a higher level of serum IGF-1. The finding of an increased frequency of the IGF-1 allele A3 in individuals with ROA and/or disk degeneration is in line with higher serum IGF-1 levels that were observed in subjects with osteophytic progression in the knee joints (10) and in women with knee OA (11). Possibly, the interactive effect between the IGF-1 and

COL2A1 loci observed in the present study could be caused by a predisposing effect of the COL2A1 gene that is only exhibited in the co-existence of allele A3 of the IGF-1 gene. One previous report suggested that the expression of the COL2A1 gene in cultured chondrocytes was stimulated by IGF-1 (23). High serum IGF-1 levels may parallel high tissue IGF-1 levels, which may stimulate chondrocytes, for example in response to cartilage damage, to overexpression in case COL2A1 allele 13R1 is present. This may lead to an inappropriate production of collagen type II fibers that can not be incorporated in the extracellular cartilage network (24). This may result eventually in a more rapid progression of OA.

In conclusion the present study suggests an interactive effect between the IGF-1 and COL2A1 genes, through a mechanism in which the prevalence of ROA and/or disk degeneration was only increased in individuals carrying the risk alleles at both loci. The allele of the IGF-1 gene responsible for this interaction has previously been associated with a higher serum IGF-1 level, suggesting that high levels of IGF-1 are associated with the pathogenesis of OA.

References

1. Spector TD, Cicuttini F, Baker J, Loughlin J, Hart D. Genetic influences on osteoarthritis in women: a twin study. BMJ 1996;312:940-3.
2. Felson DT, Couropmitree NN, Chaisson CE, Hannan MT, Zhang Y, McAlindon TE, et al. Evidence for a mendelian gene in a segregation analysis of generalized radiographic osteoarthritis. Arthritis Rheum 1998;41:1064-71.
3. Hirsch R, Lethbridge-Cejku M, Hanson R, Scott WW, Reichle R, Plato CC, Tobin JD, Hochberg MC. Familial aggregation of osteoarthritis. Arthritis Rheum 1998;41:1227-32.
4. Ritvaniemi P, Korkko J, Bonaventure J, Vikkula M, Hyland J, Paassilta P, et al. Identification of COL2A1 gene mutations in patients with chondrodysplasias and familial osteoarthritis. Arthritis Rheum 1995;38:999-1004.
5. Meulenbelt I, Bijkerk C, Miedema HS, Breedveld FC, Hofman A, Valkenburg HA, Pols HAP, Slagboom PE, van Duijn CM. A genetic association study of the IGF-1 gene and radiological osteoarthritis (ROA) in a population-based cohort study (the Rotterdam Study). Ann Rheum Dis 1998;57:371-4.
6. Rosen CJ, Kurland ES, Vereault D, Adler RA, Rackoff PJ, Craig WY, Witte S, Rogers J, Bilezikian JP. Association between serum insulin growth factor-1 (IGF-1) and a simple sequence repeat in IGF-1 gene: implications for genetic studies of bone mineral density. J Clin Endocrinol Metab 1998;83:2286-90.
7. LeRoith D. Insulin-like growth factors in health and disease. Ann Intern Med 1992;116:854-862.
8. McQuillan DJ, Handley CJ, Campbell MA, Bolis S, Milway VE, Herington AC. Stimulation of proteoglycan biosynthesis by serum and insulin-like growth factor-1 in cultured bovine articular cartilage. Biochem J 1986;240:423-430.
9. Rosen CJ, Donahue LR, Hunter SJ. Insulin-like growth factors and bone: the osteoporosis connection. Proc Soc Exp Biol Med 1994;206:83-102.

10. Schouten JSAG, Van den Ouweland FA, Valkenburg HA, Lamberts SWJ. Insulin-like growth factor-1: a prognostic factor of knee osteoarthritis. Br J Rheumatol 1993;32:274-280.
11. Lloyd ME, Hart DJ, Nandra D, McAlindon TE, Wheeler M, Doyle DV, Spector TD. Relation between insulin-like growth factor-I concentrations, osteoarthritis, bone density, and fractures in the general population: the Chingford study. Ann Rheum Dis 1996;55:870-4.
12. Hochberg MC, Lethbridge-Cejku M, Scott WW, Reichle R, Plato CC, Tobin JD. Serum concentrations of insulin-like growth factor 1 in subjects with osteoarthritis of the knee. Arthritis Rheum 1994;37:1177-80.
13. Denko CW, Boja B, Moskowitz RW. Growth promoting peptides in osteoarthritis: insulin, insulin-like growth factor-1, growth hormone. J Rheumatol 1990;17:1217-21.
14. Palotie A, Ott J, Elim K, Cheah K, Vaisanen P, Ryhanen L, et al. Predisposition to familial osteoarthrosis linked to type II collagen gene. Lancet 1989;i:924-7.
15. Hofman A, Grobbee DE, De Jong PVTM, van den Ouweland FA. Determinants of disease and disability in the elderly. Eur J Epidemiol 1991;7:403-22.
16. Kellgren JH, Jeffrey MR, Ball J. (editors). 1963. The epidemiology of chronic rheumatism. Volume II: Atlas of standard radiographs of arthritis. Oxford: Blackwell Scientific Publications.
17. Odding E, Valkenburg HA, Algra D, van den Ouweland FA, Grobbee DE, Hofman A. Associations of radiological osteoarthritis of the hip and knee with locomotor disability in the Rotterdam Study. Ann Rheum Dis 1998;57:203-8.
18. Burger H, van Daele PLA, Odding E, Valkenburg HA, Hofman A, Grobbee DE, et al. Osteoarthritis is associated with higher bone mineral density and increased bone loss with age: The Rotterdam Study. Arthritis Rheum 1996;39:81-6.
19. Berg ES, Olaisen B. Characterization of the COL2A1 VNTR polymorphism. Genomics 1993;16:350-4.
20. Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 1989;44:388-396.
21. Uitterlinden AG, Burger H, Huang Q, Odding E, van Duijn CM, Hofman A, Birkenhäger JC, van Leeuwen JPTM, Pols HAP. Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J Clin Invest 1997;100:259-63.
22. Eisman JA. Vitamin D receptor gene alleles and osteoporosis: an affirmative view. J Bone Miner Res 1995;10:1289-93.
23. Sandell LJ, Dudek E. IGF-1 stimulates type II collagen gene expression in cultured chondrocytes (abstract). Orthop Trans 1988;13:300.
24. Matyas JR, Adams ME, Huang D, Sandell LJ. Discoordinate gene expression of aggrecan and type II collagen in experimental osteoarthritis. Arthritis Rheum 1995;38:420-5.

6

General Discussion

Genetic epidemiological approach to studying osteoarthritis

In this chapter, the main findings of the present thesis will be discussed in light of previous findings reported in the literature. As this thesis primarily concerns radiological osteoarthritis (ROA) in the general population, the general discussion will focus on the association between genetic factors and radiological osteoarthritis. Furthermore, special attention is addressed to methodological issues in genetic epidemiological studies. Recommendations for future research and developments in osteoarthritis (OA) research will be discussed.

Findings and methodological considerations

Determinants of generalized radiological osteoarthritis

The findings of the present thesis support the existence of a subset of polyarticular ROA in women, meaning that clustering was found of ROA and disk degeneration at different sites (see Chapter 3.1). In women, age, body mass index, bone mineral density, and Heberden's nodes were all statistically significant risk factors of polyarticular ROA. For women the findings of the present study are consistent with previous studies (1, 2). To our knowledge this thesis is the first study to suggest that disk degeneration can be included in the concept of polyarticular ROA in women. The associations observed in women are in keeping

with a genetically determined generalized susceptibility for cartilage degradation, which may be modified by the effects of systemic factors like body mass index and bone mineral density (3).

In men, only hand ROA at multiple sites was associated with disk degeneration of the spine. Only age and body mass index were statistically significantly associated with polyarticular ROA in men (see Chapter 3.1). One earlier study found evidence for polyarticular disease in men, based on the statistically significant association of hand and knee ROA (1).

The finding in both men and women that disk degeneration of the spine is associated with hand ROA confirms the findings of an experimental study (4) and suggests a shared, possibly genetic, pathogenesis of ROA and disk degeneration (5). An important implication for genetic studies of this finding is that disk degeneration of the spine should be included in the concept of polyarticular ROA. Disk degeneration may be regarded the progressive deterioration of hyaline cartilage of the intervertebral disk. A shared pathogenesis in ROA and disk degeneration could for example be explained by changes in the collagen network (e.g. collagen types II, IX, X or XI), the extracellular proteoglycan metabolism, the chondrocyte function or function of proteolytic enzymes or cytokines (6).

Heritability of OA

The findings of this thesis show that hand ROA and disk degeneration of the spine in the general population are largely determined by genetic factors, with heritability estimates of respectively 0.56 (95 % CI 0.34-0.76) and 0.75 (95 % CI 0.30-1.00) (see Chapter 3.2). These results were detected independent of the effects of age, sex, body mass index and bone mineral density. That OA is a heritable disorder was already recognized by Stecher in the 1940's (7), and confirmed in the 1950's, in studies on generalized OA, by Kellgren and Lawrence (8). Recent population-based studies have shown results for sib-sib correlations in hand ROA, knee ROA and the combination of hand and knee ROA, that are largely similar to the results presented in this thesis, as is outlined in Table 1 (9-11). The presented correlations were in all studies at least adjusted for age and weight. In our study, no statistically significant genetic influence was observed on the variance of knee ROA in the general population. Moreover, in this thesis no genetic effect was observed on the occurrence of hip ROA in the general population (see Chapter 3.2). However, a genetic influence on severe hip OA leading to joint replacement has been reported (12). No earlier data have been published concerning the heritability of disk degeneration of the spine, although one recent report suggests a genetic susceptibility to this disorder based on an observed association with the Vitamin D receptor gene (13). In view of possible linkage disequilibrium between the VDR and COL2A1 genes, it is not clear

Table 1
Comparison of sib-sib correlations between the present study and three previous studies (a twin study and two population-based studies).

Study	Spector et al. (9) 1996	Felson et al. (10) 1998	Hirsch et al. (11) 1998	Present study
Sib-sib correlation hand ROA	0.24†	not given	0.65*	0.30*
Sib-sib correlation knee ROA	0.17†	not given	0.09‡	0.09‡
Sib-sib correlation hand and knee ROA	0.20†	0.31†	0.23‡	0.31*
Definition of ROA	Burnett et al. (42)	K & L (43)	K & L (43)	K & L (43)
Type of study	dizygotic twins	random population	community volunteers	random population
Statistical adjustments	age, weight	age, sex, BMI, PAI, multiple comparisons	age, sex, BMI	age, sex, BMI, BMD

ROA = radiological osteoarthritis. BMI = body mass index. PAI = physical activity index. BMD = bone mineral density. K & L = Kellgren and Lawrence. * $P < 0.001$. † P-value not given. ‡ Not significant.

which gene actually underlies the observed association. In our study, we observed the highest heritability estimate for a score summing the total number of joints affected with ROA and/or disk degeneration (0.78 with 95 % CI 0.52-0.98), further supporting the evidence for a shared genetic etiology of both conditions (4, 5).

Radiological osteoarthritis and collagen genes

The collagen network is essential for the dynamic mechanical and structural integrity of articular cartilage. The main constituents are collagen type II, about 90 percent of the collagen content of cartilage, collagen type IX, forming the cross links between the type II fibrils, and collagen type XI, regulating the diameter of the type II fibrils. Collagen type II consists of three similar alpha chains encoded by the COL2A1 gene on chromosome 12. Collagen type IX and XI, both consist of three separate alpha chains, encoded respectively by the COL9A1, COL9A2, and COL9A3 genes and the COL2A1, COL11A1, and COL11A2 genes. Except for the COL9A3 gene, mutations have been identified for all above-mentioned genes leading to some form of severe, mostly early-onset, familial OA (14-18).

COL2A1 gene

– *Association study* This thesis describes the association of the most frequent allele (allele frequency 0.42) of a VNTR polymorphism 1.35 kb to the 3' end of the COL2A1 gene with ROA (see Chapter 4.1). The strongest effects were observed in women homozygous for this allele 13R1 (OR = 1.86, 95 % CI 1.0-3.6). The ORs for women with polyarticular OA were respectively 3.87, 95 % CI 1.1-13.3 for homozygotes and 1.59 with 95 % CI 0.6-4.1 for heterozygotes. Also the effect was stronger in women with Heberden's nodes as compared to women without Heberden's nodes. In two previous association studies concerning relatively severe OA cases, rare alleles of the COL2A1 gene were statistically significantly associated with the occurrence of ROA (19, 20). Two other association studies, respectively carried out in Finnish cases with generalized OA or hand OA and in Belgian hip OA cases, did not find evidence for an association of OA with the COL2A1 gene (21, 22). This thesis provides the first report of an association between common forms of ROA and the COL2A1 gene in a population-based study. Given the cross-sectional design of the present study, the observed association with the COL2A1 VNTR locus gives no clues about the biological importance of this finding in terms of the etiology or prognosis of OA.

– *Sibling pair study* We performed a sibling pair study with probands, derived from the above-mentioned population-based association study (Chapter 4.1), and their corresponding siblings. For the COL2A1 gene, the findings of the sibling pair study were in agreement with the association reported in Chapter 4.1,

although no statistically significant results were found (Chapter 4.3). For sibling pairs of probands carrying the 13R1 allele, a marked decrease of the variance in a score summing the total number of joints affected with ROA and disk degeneration was observed when siblings shared one or two alleles (σ^2_0 = 16.2, σ^2_1 = 10.0 and σ^2_2 = 6.7, with LOD score 0.2). One earlier sibling pair study of limited size (38 sibling pairs), examining siblings with three or more joint sites affected, failed to show linkage to the COL2A1 locus (23). In a recent unpublished report, concerning a sibling pair study, derived from 52 extended families, in the Framingham Study, no evidence for linkage was found between the COL2A1 locus and ROA (24). This study was carried out with five microsatellite markers flanking the COL2A1 locus. Each of these studies, including our study, lacked statistical power for showing a significant result.

– *Interaction with the insulin-like growth factor-1 (IGF-1) gene* The IGF-1 gene is also a candidate gene for OA and was found to be associated with ROA (Chapter 5.1). Allele A3 of a polymorphism in the promotor region of the IGF-1 gene was associated with ROA (OR for heterozygous subjects was 1.9, 95 % CI 1.2-3.1 and for homozygous subjects 3.6, 95 % CI 0.8-16.2), independent of the influences of bone mineral density and body mass index (25).

We observed an interactive effect on the presence of ROA between the IGF-1 gene and the COL2A1 gene (Chapter 5.2). This study showed that COL2A1 VNTR allele 13R1 is only associated with ROA in the presence of allele A3 of the IGF-1 gene (OR = 2.0, 95 % CI 1.0-3.9 for individuals heterozygous for allele 13R1, and OR = 4.7, 95 % CI 1.0-21.0 for individuals homozygous for allele 13R1). Alternatively, no association was observed between IGF-1 allele A3 and ROA in the absence of COL2A1 VNTR allele 13R1. This clearly suggests the existence of an epistatic effect of both loci on the occurrence of ROA.

– *COL2A1/VDR locus* The Vitamin D receptor (VDR) gene, located about 750 kb distant from the COL2A1 gene, was found to be associated with disk degeneration (13), knee ROA (26, 27), and bone mineral density (28). Given the distance between both genes linkage disequilibrium may exist, raising the question whether the COL2A1 gene itself contributes to the etiology of ROA or the VDR gene instead. The observed association between the COL2A1 VNTR polymorphism and ROA (Chapter 4.1) and the interactive effect of this locus with the IGF-1 gene on the occurrence of ROA (Chapter 5.2) was independent of bone mineral density (BMD). This suggests that it is unlikely that the VDR gene underlies the observed association through its effect on BMD.

– *Genomic screens* Recent reports, concerning two genomic screens in respectively Finnish sibships from a genetic isolate, and affected sibling pairs who

had underwent joint replacement, showed no evidence for linkage to chromosome 12 (the localization of the COL2A1/VDR locus) (29, 30). In the genetically isolated Finnish population genetic drift could explain the finding that the COL2A1 gene does not play a role in the etiology of OA. The second genomic screen was carried out in British patients who underwent joint replacements which represents severe cases of principally hip OA, that could well be a OA phenotype with an etiology in which the type II collagen is not involved. In our heritability studies hip ROA was not highly genetic. Therefore, in the present study of ROA, in contrast to studies of hip replacements, a different phenotype was studied.

- *Conclusion* This thesis suggests a role of the COL2A1 locus in the etiology of common forms of OA, by means of an association study. The findings of the sibling pair study subsequently carried out in the same study population were in line with these findings. We observed that this association of the COL2A1 locus with ROA was fully determined by the influence of the IGF-1 gene. Such an epistatic model will reduce the power of past and future sibling pair studies concerning the COL2A1 gene, unless the effect of both genes is studied simultaneously. Although, we can not exclude the possibility that the adjacent VDR gene is contributing to the detected association, we can conclude that it will not be through an effect of the VDR gene on bone mineral density.

COL9A1 gene

We observed an association between a Short Tandem Repeat Polymorphism (STRP) within the COL9A1 gene and ROA in women from a population-based sample (Chapter 4.2). Two distinct haplotypes were detected that showed respectively a three ($p = 0.01$) and a seven ($p < 0.002$) times decreased frequency in women with ROA. This observed association could not be confirmed in the sibling pair study with probands who were affected with ROA at two or more joint sites (Chapter 4.3). However, the present sibling pair study mainly included affected siblings (only 12 siblings were free from ROA and disk degeneration), therefore it seems likely that in this sample it was not possible to confirm the observed protective effect, because of a lack of statistical power. For this gene no previous association or sibling pair studies have been published. Given the absence of consistency between our association study and sibling pair study, the role of the COL9A1 gene in the etiology of OA remains to be confirmed.

COL11A2 gene

Since no intragenic markers were available, this gene was studied indirectly through the use of two flanking markers; tumor necrosis factor a (TNFa) within the TNF-ß gene (1.5 cM from the COL11A2 gene) and a dinucleotide repeat

marker (D6S291) at a 2 cM distance from the COL11A2 gene. The COL11A2 gene is located within the major histocompatibility complex (MHC) on chromosome 6, just as the TNF-ß gene. The MHC region exhibits a very low recombination fraction and therefore TNFa and D6S291 can be used in a haplotype analysis of the COL11A2 gene. Furthermore, the TNF-α gene, directly adjacent to the TNF-ß gene was considered a candidate gene for OA itself, given the role of TNF-α in the pathophysiology of cartilage degradation (31). Both genes have not been studied before in OA in humans. No association was found between the COL11A2 gene or the TNF-α gene and the presence of ROA in the general population (Chapter 4.2).

Strategies for future research

Family-based studies

The role of classical linkage studies in detecting disease-susceptibility loci in common forms of OA is expected to be limited, given the multifactorial nature of the disease and the subsequent difficulty of detecting extended families with affected pedigree members. Classical linkage studies remain important for detecting causative mutations in early-onset, mostly more severe, OA phenotypes.

OA is a multifactorial disease that is caused by multiple genes interacting with each other and with environmental factors, which creates a gradient of genetic susceptibility to disease. The degree and type of interaction between these genes influences the chances of detecting genes through a sibling pair analysis (32). Another factor determining the successfulness of sibling pair studies is the definition of the phenotype under investigation. Sibling pair methods can be applied in both a candidate gene approach and random genome scans, although it has been suggested that in a candidate gene approach association studies are to be preferred (33). A sibling pair approach considering OA a quantitative trait may render a high power for detecting disease-susceptibility loci as the heritability of this trait is high (see Chapter 3.2) and both affected and unaffected relatives can be included in the study (34). The sum score of the total number of joint sites affected with ROA in peripheral joints and disk degeneration of the spine was used in this thesis and was shown to be determined largely by genetic influences (Chapter 3.2). Concerning the statistical power of a study, it has been shown that, for a common trait, the use of the population distribution of a phenotype is to be preferred over an affected sibling pair approach (35). Recruitment of sibling pairs from both extremes of a phenotype distribution, e.g. highest versus lowest decile, may be an alternative powerful approach (36). How-

ever, the usefulness of the extreme discordant sibling pair approach in practice needs to be established.

Recently, two unpublished reports concerning genomic screens in affected sibling pairs with relatively rare OA phenotypes showed excess sharing in OA patients of chromosomal regions on chromosome 2q and 11q (29, 30). Candidate genes located in these regions are the interleukin-1 cluster and the cluster of genes encoding matrix metalloproteases. The relevance of these findings needs to be established with use of a dense set of marker loci in these chromosomal regions. Further genomic screens in sibling pairs with more common forms of OA are needed in order to obtain a more complete view on susceptibility loci for OA. The availability of a genome-wide high-resolution map (0.5-1.0 Mb) will increase the possibilities for fine mapping and eventually positional cloning of a disease-susceptibility locus (37, 38).

Association studies

At present, only a candidate gene approach is feasible in unrelated individuals, except for genomic screens that are carried out in genetically isolated populations. Population stratification is the major confounding factor in association studies and can be due either to recent admixture of different populations or to inappropriate matching of patients and controls (39, 40). Given this possibility of confounding, findings need to be confirmed using a transmission/disequilibrium test (TDT) or using haplotype analysis (41). However, the TDT is not a powerful approach and is practically difficult in late-onset diseases. An important issue for future genetic epidemiological studies will be to quantify the presence of population stratification. Association studies are powerful as a follow-up strategy for examining disease-susceptibility loci that have been identified in genomic screens or linkage studies as long as these studies are carried out in well-defined large homogeneous populations to reduce confounding and information bias.

Population relevance

OA is considered a genetically heterogeneous disorder in which several genes act in interaction with each other, either in a multiplicative or in an additive model. In this way genetically susceptible individuals are characterized by a distinct set of genes that give rise to articular cartilage more prone to progressive degeneration. Different individuals have different sets of genes leading to the same phenotype. In future, large-scale population-based studies are needed to establish which genes demonstrate an interactive effect on the development or progression of OA.

Finally, it is important to realize that data derived from association and sibling pair studies and from genomic screens only provide indirect evidence for

the role of a gene or a locus in the etiology of OA. In case of non-coding sequences that are found to be associated with OA in genomic screens or a candidate gene study, positional cloning and mutation analysis should indicate which mutation is responsible for the observed association. Also, further molecular genetic studies in affected subjects, cell and animal models are needed to determine through what mechanism a mutation, e.g. leading to a causative protein isoform or an altered gene expression, is involved in causing OA. The final test for the relevance of a mutation in the occurrence of OA is to determine the associated risk of disease in a prospective follow-up study. Accordingly, the studies presented in this thesis are to be interpreted as the first step in unraveling the genetically determined pathogenesis of OA.

References

1. Hirsch R, Lethbridge-Cejku M, Scott Jr WW, Reichle R, Plato CC, Tobin J, Hochberg MC. Association of hand and knee osteoarthritis: evidence for a polyarticular disease subset. Ann Rheum Dis 1996;55:25-9.
2. Cooper C, Egger P, Coggon D, Hart DJ, Masud T, Cicuttini F, Doyle DV, Spector TD. Generalized osteoarthritis in women: pattern of joint involvement and approaches to definition for epidemiological studies. J Rheumatol 1996;23:1938-42.
3. Cicuttini FM, Spector TD. Genetics of osteoarthritis. Ann Rheum Dis 1996;55:665-7.
4. Kimura T, Nakata K, Tsumaki N, Miyamoto S, Matsui Y, Ebara S, Ochi T. Progressive degeneration of articular cartilage and intervertebral discs. An experimental study in transgenic mice bearing a type IX collagen mutation. Int Orthop 1996;20:177-81.
5. Matsui H, Kuramori M, Ishihara H, Yudoh K, Naruse Y, Tsuji H. Familial predisposition for lumbar degenerative disc disease. A case-control study. Spine 1998;23:1029-34.
6. Bleasel JF, Holderbaum D, Brancolini V, Moskowitz RW, Considine EL, Prockop DJ, Devoto M, Williams CJ. Five families with arginine 519-cysteine mutation in COL2A1: evidence for three distinct founders. Hum Mutat 1998;12:172-6.
7. Stecher RM. Heberden's nodes. Heredity in hypertrophic arthritis of the finger joints. Am J Med Science 1941;201:801.
8. Kellgren JH, Lawrence JS, Bier F. Genetic factors in generalized osteoarthrosis. Ann Rheum Dis 1963;22:237-55.
9. Spector TD, Cicuttini F, Baker J, Loughlin J, Hart D. Genetic influences on osteoarthritis in women: a twin study. BMJ 1996;312:940-3.
10. Felson DT, Couropmitree NN, Chaisson CE, Hannan MT, Zhang Y, McAlindon TE, LaValley M, Levy D, Myers RH. Evidence for a mendelian gene in a segregation analysis of generalized radiographic osteoarthritis. Arthritis Rheum 1998;41:1064-71.
11. Hirsch R, Lethbridge-Cejku M, Hanson R, Scott WW, Reichle R, Plato CC, Tobin JD, Hochberg MC. Familial aggregation of osteoarthritis. Arthritis Rheum 1998;41:1227-32.
12. Chitnavis J, Sinsheimer JS, Clipsham K, Loughlin J, Sykes B, Burge PD, Carr AJ. Genetic influences in end-stage osteoarthritis. Siblings risks of hip and knee replacements for idiopathic osteoarthritis. J Bone Joint Surg Br 1997;79:660-4.

13. Videman T, Leppavuori J, Kaprio J, Battie MC, Gibbons LE, Peltonen L, Koskenvuo M. Intragenic polymorphisms of the Vitamin D receptor gene asociated with intervertebral disc degeneration. Spine 1998;23:2477-85.
14. Spranger J, Winterpacht A, Zabel B. The type II collagenopathies: a spectrum of chondrodysplasias. Eur J Pediatr 1994;153:56-65.
15. Nakata K, Ono K, Miyazaki JI, Olsen BR, Muragaki Y, Adachi E, Yamamura KI, Kimura T. Osteoarthritis associated with mild chondrodysplasia in transgenic mice expressing α1(IX) collagen chains with a central deletion. Proc Natl Acad Sci USA 1993;2870-4.
16. Muragaki Y, Mariman ECM, van Beersum SEC, Perälä M, van Mourik JB, Warman ML, Olsen BR, Hamel BCJ. A mutation in the gene encoding the α2 chain of the fibril-associated collagen IX, COL9A2, causes multiple epiphyseal dysplasia (EDM2). Nat Genet 1996;1:103-5.
17. Brunner HG, van Beersum SEC, Warman ML, Olsen BR, Ropers HH, Mariman ECM. A Stickler syndrome gene is linked to chromosome 6 near the COL11A2 gene. Hum Mol Genet 1994;3:1561-4.
18. Richard AJ, Yates JRW, Williams R, Payne SJ, Pope FM, Scott JD, Snead MP. A family with Stickler syndrome type 2 has a mutation in the COL11A1 gene resulting in the substitution of glycine 97 by valine in α1 (XI) collagen. Hum Mol Genet 1996;5:1339-43.
19. Hull R, Pope FM. Osteoarthritis and cartilage collagen genes. Lancet 1989;i:1337-8.
20. Loughlin J, Irven C, Athanasou N, Carr A, Sykes B. Differential allelic expression of the type II collagen gene (COL2A1) in osteoarthritic cartilage. Am J Hum Genet 1995;56:1186-93.
21. Vikkula M, Nissilä M, Hirvensalo E, Nuotio P, Palotie A, Aho K, Peltonen L. Multiallelic polymorphism of the cartilage collagen gene: no association with osteoarthritis. Ann Rheum Dis 1993;52:762-4.
22. Aerssens J, Dequeker J, Peeters J, Breemans S, Boonen S. Lack of association between osteoarthritis of the hip and gene polymorphisms of VDR, COL1A1, COL2A1 in postmenopausal women. Arthritis Rheum 1998;41:1946-50.
23. Loughlin J, Irven C, Fergusson C, Sykes B. Sibling pair analysis shows no linkage of generalized osteoarthritis to the loci encoding type II collagen, cartilage link protein or cartilage matrix protein. Br J Rheumatol 1994;33:1103-6.
24. Baldwin CT, Joost O, Chaisson C, McAlindon T, Farrer L, Ordovas J, Schaefer E, Levy D, Myers R, Felson D. The type II collagen/Vitamin D receptor locus and osteoarthritis: The Framingham Osteoarthritis Study. Arthritis Rheum 1998;41:S352.
25. Meulenbelt I, Bijkerk C, Miedema HS, Breedveld FC, Hofman A, Valkenburg HA, Pols HAP, Slagboom PE, van Duijn CM. A genetic association study of the IGF-1 gene and radiological osteoarthritis in a population-based cohort study (the Rotterdam Study). Ann Rheum Dis 1998;57:371-4.
26. Uitterlinden AG, Burger H, Huang Q, Odding E, van Duijn CM, Hofman A, Birkenäger JC, van Leeuwen JPTM, Pols HAP. Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J Clin Invest 1997;100:259-63.
27. Keen RW, Hart DJ, Lanchbury JS, Spector TD. Association of early osteoarthritis of the knee with a Taq 1 polymorphism of the Vitamin D receptor gene. Arthritis Rheum 1997;40:1444-49.
28. Eisman JA. Vitamin D receptor gene alleles and osteoporosis: an affirmative view. J Bone Miner Res 1995;10:1289-93.
29. Leppävuori JK, Kujala UM, Kaprio J, Nissilä M, Heliövaara M, Kinnunen J, Koskenvuo M. Genome scan for predisposing loci of dital interphalangeal joint osteoarthritis. Am J Hum Genet 1998;63:A297.
30. Chapman K, Mustafa Z, Irven C, Smith A, Carr A, Clipsham K, Chitnavis J, Sinsheimer J, Sykes B, Loughlin J. A comprehensive genome-wide screen for susceptibility loci in osteoarthritis: linkage to chromosomes 2q and 11q. Am J Hum Genet 1998;63:A16.

31. Saklatvala J. Tumour necrosis factor ∀ stimulates resorption and inhibits synthesis of proteoglycan in cartilage. Nature 1986;322:547-59.
32. Eaves LJ. Effect of genetic architecture on the power of human linkage studies to resolve the contribution of quantitative trait loci. Heredity 1994;72:175-92.
33. Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science 1996;273:1516-7.
34. Weeks DE, Lathrop GM. Polygenic disease: methods for mapping complex disease traits. Trends Genet 1995;11:513-9.
35. Wijsman EM, Amos CI. Genetic analysis of simulated oligogenic traits in nuclear and extended pedigrees: Summary of GAW10 contributions. Genet Epidemiol 1997;14: 719-35.
36. Risch N, Zhang H. Extreme discordant sib-pairs for mapping quantitative trait loci in humans. Science 1995;268:1584-9.
37. Collins FS. Positional cloning moves from perditional to traditional. Nat Genet 1995;9:347-50.
38. Walter MA, Spillet DJ, Thomas P, Weissenbach J, Goodfellow PN. A method for constructing radiation hybrid maps of whole genomes. Nat Genet 1994;7:22-8.
39. Knowler WC, Williams RC, Pettitt DJ, Steinberg AG. Gm3;5,13,14 and type 2 diabetes mellitus: an association in American Indians with genetic admixture. Am J Hum Genet 1988;43:520-6.
40. Lander ES, Schork NJ. Genetic dissection of complex traits. Science 1994;265:2037-48.
41. Spielman RS, McGinnis RE, Ewens WJ. The transmission/disequilibrium test detects segregation and linkage. Am J Hum Genet 1994;54:559-60.
42. Burnett S, Hart DJ, Cooper C, Spector TD. A radiographic atlas of osteoarthritis. Springer-Verlag, London. 1994.
43. Kellgren JH, Jeffrey MR, Ball J. (editors). The epidemiology of chronic rheumatism. Volume II: Atlas of standard radiographs of arthritis. Oxford: Blackwell Scientific Publications. 1963.

7

Summary

This thesis describes an approach of population-based genetic association studies and sibling pair studies to study common forms of osteoarthritis (OA) occurring in the general population. OA is characterized by a progressive degeneration of articular cartilage of diarthrodial joints and has a multifactorial etiology. The genetic influence on the etiology of OA has been recognized for familial forms of severe early-onset OA, which is often associated with osteochondrodysplasia. There is growing evidence from population-based studies that common late-onset forms of OA are also heritable. However, at present it is unclear which genes are involved in causing these common forms of OA in the elderly.

First, some methodological considerations concerning genetic epidemiological studies on OA are presented (**chapter 2.1**). A candidate gene approach is powerful in case a genetic association study is applied that was conducted in an unselected population. However, findings of these association studies need confirmation in either a sibling pair study or in a haplotype analysis. Genomic screens can be conducted based on the findings of previous studies or can scan the entire genome for susceptibility loci. Bias resulting from multiple testing is an important issue of consideration in every genetic study. Population stratification or admixture is the main source of bias in association studies. Sibling pair studies in late-onset complex traits are usually hampered by a lack of statistical power, because of difficulties in ascertaining sufficient numbers of siblings.

For the results presented in this thesis, data were used from a random sample derived from the Rotterdam Study (**chapter 2.2**) and from a sample of sibling pairs (**chapter 2.3**). The Rotterdam Study is a population-based cohort study among all inhabitants, aged 55 years and over, of the suburb Ommoord in Rotterdam. In total, 7983 individuals participated in the Rotterdam Study (response 78%). For this thesis a random sample was drawn of 1583 individuals aged 55 to 70 years. All individuals visited the research center where interviews were held and extensive measurements were performed, including radiographs of the knees, hips, hands, and thoracolumbar spine and dual energy X-ray absorptiometry (DEXA) scans. Two independent readers scored all radiographs for radiological OA (ROA) or disk degeneration according to the Kellgren scoring system. Individuals with at least two or more joint sites affected were selected as probands. In case only hand ROA and disk degeneration of the spine was present, individuals had to have additional Heberden's nodes to be included as probands. All available siblings, who agreed to participate, were recruited for sibling pair studies.

In **chapter 3.1** the determinants for ROA and disk degeneration at multiple sites and the clustering of ROA at all four joint sites are investigated. In women, body mass index (BMI), bone mineral density (BMD), and Heberden's nodes

were besides age all statistically significant risk factors of polyarticular disease and knee ROA was significantly associated with radiological abnormalities all other sites studied. In men, only BMI was in addition to age significantly associated with polyarticular disease and only polyarticular hand ROA was associated with disk degeneration of the spine. These findings support the existence of a subset of polyarticular disease in women.

Chapter 3.2 describes the genetic influence on the occurrence of ROA in the knees, hips, and hands and disk degeneration of the spine in the general population. Heritability estimates for hand ROA and disk degeneration were statistically significant, respectively 0.56 (95% CI 0.34-0.76) and 0.75 (95% CI 0.30-1.00). For knee and hip ROA no evidence for a genetic effect in the general population was found. The heritability estimate for a score summing the total number of joints affected at four different sites was 0.78 (95% CI 0.52-0.98), suggesting a genetic susceptibility to generalized OA.

The association study of the procollagen type II (COL2A1) gene with ROA is presented in **chapter 4.1.** The frequency distribution of alleles of the COL2A1 VNTR polymorphism, which is located 1.35 kb to the COL2A1 gene, in female ROA cases differed statistically significantly from that in female referents ($p = 0.03$). This was explained by an increased frequency of the most common allele 13R1 (adjusted OR for female cases heterozygous for allele 13R1 was 1.71, 95% CI 1.06-2.76 and for female cases homozygous 1.86, 95% CI 0.95-3.64). Strongest effects were found in women with ROA and Heberden's nodes and in women with polyarticular ROA.

In **chapter 4.2** the association study concerning the genes encoding the α-1 chain of collagen type IX (COL9A1) and the α-2 chain of collagen type XI (COL11A2) and ROA is described. For the COL9A1 gene, one of two Short Tandem Repeat Polymorphisms (STRP 509-12B1), within the COL9A1 gene, was found significantly associated with ROA in women ($p = 0.002$). Haplotype analysis suggested a protective effect of allele 12B1-A2, with a seven times decreased frequency in female ROA cases. No evidence was observed for a role of the COL11A2 gene in the occurrence of ROA.

In **chapter 4.3** data is presented on a sibling pair study that was performed in 257 siblings of 118 probands, derived from the Rotterdam Study, with ROA and disk degeneration at multiple sites. For the COL2A1 locus, a reduction was observed in the variance for the sum score of ROA and disk degeneration and for the sum score of hand ROA (quantitative trait loci), with an increase in the number of alleles shared between female siblings (not statistically significant). Effects were stronger for women with ROA and Heberden's nodes and for women with generalized ROA. We could not find evidence that the COL9A1 locus increases the risk of developing ROA or disk degeneration.

The third association study in this thesis concerns the insulin-like growth factor 1 (IGF-1) gene (**chapter 5.1**). The IGF-1 locus was statistically significantly associated with the presence of ROA and /or disk degeneration. This association was explained by an increased frequency of IGF-1 allele A3 in affected subjects (adjusted OR for heterozygous subjects was 1.9, 95% CI 1.2-3.1 and for homozygous subjects 3.6, 95% CI 0.8-16.2).

In **chapter 5.2** the findings are reported of a study investigating the possible interaction between the IGF-1 and COL2A1 genes on the occurrence of ROA and disk degeneration. The prevalence of ROA and/or disk degeneration was significantly increased in individuals who carry both the IGF-1 allele A3 and COL2A1 allele 13R1 as compared to individuals carrying none of these alleles (adjusted OR = 2.2, 95% CI 1.1-4.3). There was no evidence for an effect of COL2A1 allele 13R1 in the absence of the IGF-1 allele A3 or for an effect of the IGF-1 allele A3 in the absence of COL2A1 allele 13R1. The findings suggests that the IGF-1 and COL2A1 genes are involved in the occurrence of ROA and/or disk degeneration of the spine through a mechanism in which both genes interact.

Chapter 6 summarizes our findings together with observations from other studies and methodological considerations. It is shown that the evidence of a genetic influence on the occurrence of common forms of OA in the general population is conclusive. The discussion focuses on the contribution of the COL2A1 gene in this genetic influence. In light of previous and present findings, a role of the COL2A1 locus is very likely. Furthermore, this influence of the COL2A1 locus is fully determined by the influence of the IGF-1 locus, possibly through an effect on the expression of the COL2A1 gene. Finally, recommendations for future research are given.

8

Samenvatting

Dit proefschrift beschrijft een benadering, gebaseerd op genetische associatiestudies en broer-zuster studies, tot het bestuderen van gewone vormen van artrose zoals die optreden in de algemene bevolking. Artrose wordt gekarakteriseerd door een progressieve degeneratie van gewrichtskraakbeen van diarthrodale gewrichten en heeft een multifactoriële etiologie. Een rol van genetische factoren in de etiologie van artrose is aangetoond voor familiaire vormen van ernstige vroegtijdige artrose, die vaak gepaard gaat met chondrodysplasie. Er bestaat toenemend bewijs afkomstig van bevolkingsonderzoeken dat ook gewone vormen van laat optredende artrose erfelijk bepaald zijn. Tot op heden is het niet duidelijk welke genen betrokken zijn bij het ontstaan van deze gewone vormen van artrose bij ouderen.

Ter introductie worden enkele methodologische aspecten met betrekking tot genetisch epidemiologische studies naar artrose besproken (**hoofdstuk 2.1**). Een kandidaat gen benadering kan succesvol zijn indien dit wordt uitgevoerd binnen een genetische associatie studie in een niet geselecteerde populatie. De resultaten van zo een associatie studie dienen bevestigd te worden in ofwel een broer-zuster studie ofwel een haplotype analyse. Een genoom scan kan worden verricht gebaseerd op de bevindingen afkomstig van vroegere studies of het gehele genoom kan worden gescand op zoek naar regio's die het risico op ziekte beïnvloeden. Het optreden van vertekening van de resultaten door het uitvoeren van herhaalde statistische testen is een belangrijk punt van aandacht in elke genetische studie. Populatie stratificatie of vermenging is een belangrijke bron van vertekening in een genetische associatie studie. Daarentegen worden broer-zuster studies gekenmerkt door een gebrek aan statistische power doordat in het algemeen het aantal paren dat verzameld kan worden onvoldoende is.

Voor de resultaten die gepresenteerd worden in dit proefschrift werd gebruik gemaakt van gegevens afkomstig van een steekproef van het Erasmus Rotterdam Gezondheid en Ouderen (ERGO) onderzoek (**hoofdstuk 2.2**) en van een verzameling broers en zusters (**hoofdstuk 2.3**). Het ERGO-onderzoek is een longitudinaal bevolkingsonderzoek onder inwoners van 55 jaar en ouder woonachtig in de Rotterdamse wijk Ommoord. In totaal deden 7983 personen mee aan het ERGO-onderzoek (respons 78%). In het kader van dit proefschrift werd hieruit een steekproef genomen van 1583 personen tussen de 55 en 70 jaar oud. Alle deelnemende personen bezochten het onderzoekscentrum alwaar een anamnese werd afgenomen en uitgebreide metingen werden verricht, inclusief röntgenfoto's van knieën, heupen, handen en de thoracolumbale wervelkolom en botdichtheidmetingen (zogenaamde DEXA-scans). De röntgenfoto's werden beoordeeld op radiologische artrose en discus degeneratie door twee, onafhankelijke, beoordelaars met behulp van de graderingen volgens Kellgren. Personen met tenminste twee aangedane gewrichtsgroepen werden geselecteerd als

pro-banden. In het geval dat een persoon zowel radiologische handartrose als discus degeneratie van de wervelkolom had, dienden additioneel noduli van Heberden aanwezig te zijn om geïncludeerd te kunnen worden als pro-band. Alle beschikbare broers en zusters van deze pro-banden, die bereid waren aan deze studie deel te nemen, werden geïncludeerd in het onderzoek.

In **hoofdstuk 3.1** worden de determinanten van de aanwezigheid van radiologische artrose en discus degeneratie in meerdere gewrichtsgroepen en de aggregatie van radiologische artrose in de perifere gewrichten en discus degeneratie van de wervelkolom onderzocht. Bij vrouwen zijn zowel "body mass index" (BMI), botdichtheid, noduli van Heberden als leeftijd statistisch significant geassocieerd met polyartrose en was knieartrose geassocieerd met radiologische afwijkingen in alle drie andere gewrichtsgroepen. Bij mannen waren alleen BMI en leeftijd significant geassocieerd met polyartrose en was alleen handartrose geassocieerd met discus degeneratie van de wervelkolom. Deze bevindingen bevestigen het bestaan bij vrouwen van een subgroep van personen met polyartrose.

Hoofdstuk 3.2 beschrijft de genetische invloed op het voorkomen van radiologische artrose in de knieën, heupen en handen en het voorkomen van discus degeneratie van de wervelkolom in de algemene bevolking. Schattingen van de erfelijkheid van handartrose en discus degeneratie van de wervelkolom waren respectievelijk 0.56 (95 % BI 0.34-0.76) en 0.75 (95% BI 0.30-1.00), hetgeen statistisch significant was. In het geval van knieartrose en heupartrose werd geen bewijs gevonden voor een genetische invloed op het voorkomen van deze aandoeningen in de algemene bevolking. De schatting van de erfelijkheid van een score, die het totale aantal aangedane gewrichten in vier verschillende gewrichtsgroepen optelt, bedroeg 0.78 (95% BI 0.52-0.98), hetgeen een genetische susceptibiliteit voor gegeneraliseerde artrose suggereert.

De associatie studie tussen het procollageen type II (COL2A1) gen en radiologische artrose wordt gepresenteerd in **Hoofdstuk 4.1**. De distributie van de allelfrequenties van het COL2A1 VNTR (variable number of tandem repeats) polymorfisme, gelokaliseerd op een afstand van 1,35 kb van het COL2A1 gen, in vrouwen met radiologische artrose verschilde statistisch significant van de distributie in de vrouwelijke controlegroep ($p = 0.03$). Dit werd veroorzaakt door een verhoogde frequentie van het meest voorkomende allel 13R1 (geadjusteerde OR voor vrouwen met radiologische artrose heterozygoot voor allel 13R1 was 1.71, 95% BI 1.06-2.76 en voor aangedane vrouwen homozygoot voor allel 13R1 was de OR 1.86, 95% BI 0.95-3.64). De sterkste effecten werden gevonden voor vrouwen met zowel radiologische artrose als noduli van Heberden en voor vrouwen met polyartrose.

In **hoofdstuk 4.2** wordt de associatie studie tussen de genen coderend voor de α-1 keten van collageen type IX (COL9A1) en de α-2 keten van

collageen type XI (COL11A2) en radiologische artrose beschreven. Voor het COL9A1 gen was één van de twee Short Tandem Repeat Polymorphisms (STRP), namelijk 509-12B1, geassocieerd met radiologische artrose bij vrouwen ($p = 0.002$). Aanvullende haplotype analyse suggereerde een beschermend effect van allel 12B1-A2 met een zeven keer verlaagde frequentie in vrouwen met radiologische artrose ten opzichte van de vrouwelijke controle groep. Er werden geen aanwijzingen gevonden voor een invloed van het COL11A2 gen op het voorkomen van radiologische artrose.

In **hoofdstuk 4.3** worden de resultaten gepresenteerd van een broer-zuster studie bij 257 broers en zusters van 118 pro-banden met radiologische artrose en discus degeneratie afkomstig uit de ERGO-studie. Een reductie in de variantie van de som score van radiologische artrose en discus degeneratie en van de som score van radiologische handartrose (kwantitatieve uitkomsten) werd geobserveerd met een toename van het aantal allelen van het COL2A1 locus dat gedeeld werd door zusters (niet statistisch significant). Deze bevindingen waren sterker voor vrouwen met radiologische artrose en noduli van Heberden en voor vrouwen met gegeneraliseerde artrose. De resultaten duidden niet op een invloed van het COL9A1 locus op het optreden van radiologische artrose en/of discus degeneratie.

De derde associatie studie die in dit proefschrift beschreven wordt, betreft het insulin-like growth factor 1 (IGF-1) gen (**hoofdstuk 5.1**). Het IGF-1 locus was statistisch significant geassocieerd met de aanwezigheid van radiologische artrose en/of discus degeneratie van de wervelkolom. Deze associatie werd veroorzaakt door een toegenomen frequentie van het IGF-1 allel A3 in aangedane personen (geadjusteerde OR voor personen heterozygoot voor allel A3 was 1.9, 95% BI 1.2-3.1 en voor personen homozygoot voor alle A3 3.6, 95% BI 0.8-16.2).

Hoofdstuk 5.2 toont de resultaten van een studie naar de mogelijke interactie tussen het IGF-1 gen en het COL2A1 gen in het optreden van radiologische artrose en discus degeneratie. De prevalentie van radiologische artrose en/of discus degeneratie was significant verhoogd in dragers van zowel het IGF-1 allel A3 als het COL2A1 allel 13R1 in vergelijking met personen die geen van beide allelen dragen (geadjusteerde OR = 2.2, 95% BI 1.1-4.3). Er was geen aanwijzing voor een effect van het IGF-1 allel A3 in de afwezigheid van COL2A1 allel 13R1 of voor een effect van het COL2A1 allel 13R1 in de afwezigheid van IGF-1 allel A3. Deze resultaten suggereren dat zowel het IGF-1 als het COL2A1 gen betrokken zijn bij het optreden van radiologische artrose en discus degeneratie van de wervelkolom door middel van een mechanisme waarbij beide genen in interactie opereren.

Hoofdstuk 6 vat de bevindingen van het huidige proefschrift samen in het licht van eerdere bevindingen en besteedt aandacht aan enkele methodologi-

sche aspecten. Er kan worden geconcludeerd dat een belangrijke genetische invloed bestaat in het optreden van gewone vormen van artrose in de algemene bevolking. De discussie concentreert zich vervolgens rond de rol die het COL2A1 gen speelt in deze genetische invloed. Gezien de huidige bevindingen en mede in ogenschouw nemend vorige bevindingen is het zeer waarschijnlijk dat dit gen inderdaad een rol speelt. Echter, deze rol is in sterke mate afhankelijk van de invloed van een ander gen, te weten het IGF-1 gen, wellicht door een effect op de expressie van het COL2A1 gen. Ten slotte worden enkele aanbevelingen gedaan voor toekomstig onderzoek.

Epiloog

Het schrijven van een proefschrift is veel meer een gezamenlijke inspanning dan blijkt uit de uiteindelijke gedrukte vorm. In eerste instantie ben ik alle ERGO deelnemers, hun broers en zusters die aan dit onderzoek hebben meegedaan, evenals alle medewerkers van ERGO zeer erkentelijk voor hun bijdrage. Vervolgens wil ik op deze plaats stilstaan bij de personen die de totstandkoming van deze dissertatie mede mogelijk hebben gemaakt.

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Curriculum vitae

Casper Bijkerk werd geboren op 21 oktober 1964 te Breda. In 1982 behaalde hij zijn eindexamen VWO aan de Rijksscholengemeenschap te Breda. Na één jaar geneeskunde gestudeerd te hebben aan het RUCA te Antwerpen, vervolgde hij zijn medicijnenstudie aan de Vrije Universiteit te Amsterdam. Op 31 mei 1991 behaalde hij hier zijn artsexamen, waarna hij gedurende 14 maanden zijn militaire dienstplicht vervulde als elnt-arts in het Militair Revalidatie Centrum te Doorn. Van 1992 tot en met 1994 werkte hij als arts-assistent op de afdeling Interne Geneeskunde van het Onze Lieve Vrouwe Gasthuis te Amsterdam (opleider: Dr. B. Silberbusch). Van januari 1995 tot oktober 1998 was hij aangesteld als arts-onderzoeker op zowel het Instituut Epidemiologie & Biostatistiek aan de Erasmus Universiteit Rotterdam (hoofd: Prof. Dr. A. Hofman) als bij TNO Preventie & Gezondheid, Gaubius Laboratorium, te Leiden. Tijdens deze aanstelling voltooide hij de opleiding tot klinisch epidemioloog. Op 1 oktober 1998 is hij begonnen aan zijn vooropleiding Interne Geneeskunde in het Sint Elisabeth Ziekenhuis te Tilburg (opleider: Dr. C. van der Heul) in het kader van zijn opleiding tot reumatoloog bij de afdeling Reumatologie van het Leids Universitair Medisch Centrum (opleider: Prof. Dr. F.C. Breedveld).
Sinds 2 september 1994 is hij getrouwd met Sarja de Pijper. Hun dochter heet Noortje.