Generalised Osteoarthritis: from Mendelian Disorder to Complex Disease

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CHAPTER

3.2 Association of matrilin-3 polymorphisms with spinal disc degeneration and with osteoarthritis of the CMC1 joint of the hand

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Annals of the Rheumatic Diseases 2006; 65(8):1060-1066

Abstract

Seven polymorphisms in the *matrilin-3 (MATN3)* gene were previously tested for genetic association with hand osteoarthritis (OA) in an Icelandic cohort. One of the variants, involving a conserved amino acid substitution (T303M; SNP5), was related to idiopathic hand OA. T303M and two other promising polymorphisms (rs2242190; SNP3, rs8176070; SNP6) were investigated for association with radiographic and symptomatic hand OA phenotypes, as well as other heritable phenotypes. Polymorphisms were examined in two distinct cohorts of subjects: a population-based sample of the Rotterdam study (n = 809) and affected siblings from the Genetics, osteoARthritis and Progression (GARP) study (n = 382). The originally described association of T303M with the hand OA phenotype was not observed in the populations studied. In the Rotterdam sample, however, carrying the T allele of T303M conferred an odds ratio of 2.9 (95% confidence interval (CI), 1.2-7.3, P = 0.02) for spinal disc degeneration. In the GARP study, carriers of the A allele of SNP6 had an odds ratio of 2.0 (95% CI, 1.3-3.1, P = 0.004) for OA of the first carpometacarpal joint (CMC1) as compared with the Rotterdam sample as a control group. Subsequent haplotype analysis showed that a common haplotype, containing the risk allele of SNP6, conferred a significant risk in sibling pairs with CMC1 OA of 1.7 (95 % CI, 1.1-2.7, P = 0.02). These associations suggest that the *MATN3* region also determines susceptibility to spinal disc degeneration and CMC1 OA.

Introduction

Osteoarthritis (OA) is a common age-related degenerative disease of the joints characterised by gradual loss of articular cartilage. Twin and sibling pair studies have demonstrated that genetic factors play a considerable role in the aetiology of OA. Heritabilities range between 30% and 80%, depending on sex and the specific joint site (spine, hand, hip, knee or generalised) involved[1,2]. Several genome-wide scans, based on a wide variety of joint site or sex specific definitions of OA, have been undertaken and have revealed positive linkage areas, some of which contain candidate genes for the susceptibility to OA[2,3].

A scan of 329 Icelandic families with idiopathic hand OA (involvement of either or both the distal interphalangeal (DIP) and the first carpometacarpal (CMC1) joints) highlighted evidence for linkage on chromosome 2p, 4q, 3p[4]. For the chromosome 2 loci, high LOD scores were reached for patients with affected CMC1 joints and for those with OA in both CMC1 and DIP joints. Recently, the genome scan by Hunter et al. revealed separate chromosomal regions for OA of CMC1 and DIP joints, again by using a joint specific hand approach[5]. Taken together, these studies indicate that OA in different hand joints should be analysed as separate entities.

The chromosome 2p locus in the Icelandic study coincides with the matrilin-3 gene (*MATN3)* encoding a noncollagenous extracellular oligomeric matrix protein[4]. Mutations in this gene have previously shown to cause different forms of multiple epiphyseal dysplasia (MED)[6-11]. This disease is characterised by generalised dysplasia of epiphyses followed by early onset OA, mainly affecting the knee and hip joints.

Stefansson et al. explored seven polymorphisms (SNP1-6 and Indel1, nomenclature as in their paper) at the *MATN3* locus and found an association with the T allele of a conserved amino acid substitution (T303M; SNP5) among patients with either idiopathic hand OA or DIP OA or CMC1 OA (0.01) as compared with the Icelandic population (0.005)[4]. In these patients, a moderate insignificant effect was observed for the A allele of SNP6 (rs8176070)[4]. The question arises as to whether variants of a gene that is widely expressed in developing cartilage and bone[12] and highly upregulated in human osteoarthritic cartilage compared with healthy cartilage[13], increases the risk of hand OA only or whether it is also involved in other heritable OA phenotypes. In this report, we therefore investigated the findings of SNP5 in relation to hand OA in a population-based sample of the Rotterdam study and in an OA affected sibpair study (Genetics, osteoARthritis and Progression study (GARP)).

We further examined whether SNP5 and two other promising *MATN3* polymorphisms (rs2242190; SNP3 and rs8176070; SNP6) of the Icelandic report were associated with radiographic and symptomatic OA phenotypes also at other joint sites in the Rotterdam sample or the GARP study. Finally, we explored the initial associations of separate SNPs at this locus by haplotype analysis.

Subjects and methods

The Rotterdam study

The Rotterdam study, which comprises 7,983 Caucasian participants is a prospective, population-based cohort study of the determinants and prognosis of chronic diseases in the elderly[14]. The medical ethics committee of the Erasmus University Medical Centre approved the study, and informed consent was obtained from all subjects. In a random sample of unrelated subjects aged 55 to 65 years (n = 809), radiographs were scored for the presence of radiographic OA (ROA) of the knees, hips[15] , the hands and thoracocolumbar spine[15;16]. All radiographs were scored according to the Kellgren/Lawrence grading system (grades 0 to 4)[17] 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 by two or more points, or was two for one reader but one for the other, a consensus score was agreed upon. ROA of the knee was only assessed in the tibiofemoral joint. In the hands, 36 separate joints were scored comprising eight joint groups: distal interphalangeal (DIP) joints, the interphalangeal joint of the thumb, the proximal interphalangeal (PIP) joints, the metacarpalphalangeal (MCP) joints, the first carpometacarpal (CMC1) joints, the trapezoscaphoideal joints, the radionavicular joints and the 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. Instead, we assessed disc degeneration of the spine, according to a Kellgren/Lawrence scale[17], at three levels that is thoracic (Th4 to Th12), lumbar (L1 to L4 or L5), and lumbosacral (L5-S1 or L5-L6). Definite ROA at a particular joint site was defined as a Kellgren/Lawrence score of two or more[17]. In the Rotterdam sample, hand ROA at one or two hand joint groups (56% and 37%, respectively) or spinal DD at one level (61%) is already very prevalent and likely to be a part of the normal wear and tear process. To enrich the sample for subjects who may be genetically predisposed to OA, we divided subjects into quartiles of the sumscore of affected hand joint groups or spinal disc levels, respectively, based on the distribution of the complete Rotterdam sample (n = 809). Subjects with hand ROA in three or more hand joint groups (the right and left hands were considered

separately), representing the highest quartile of the population, were compared to subjects in fewer than three hand joint groups. Subjects affected by spinal DD at two or more disc levels (25% cut-off value) were compared to subjects with one or no disc levels affected.

In order to confirm previous findings, the following subanalyses for hand phenotypes were conducted. Subjects with one or more DIP joints affected on each hand were compared with subjects with fewer than one DIP joint affected on each hand; subjects in one or more CMC1 joints affected were compared to subjects with fewer than one CMC1 joint affected. In our analysis, we compared each specific case group with a specific control group, which comprises the complete Rotterdam sample, excluding that specific case group. These control groups are more robust than the rare group that is completely negative for ROA in all joint groups investigated (17%).

The GARP study (Genetics, osteoARthrosis and Progression)

The ongoing GARP study, which consists of Caucasian sibling pairs of Dutch origin affected predominantly by symptomatic OA at multiple sites, is aimed at identifying determinants of OA susceptibility and progression[18]. Probands (ages 40-70 years) and their siblings had OA at multiple joint sites of the hand or in two or more of the following joint sites (hand, spine (cervical or lumbar), knee or hip[18].

Subjects with symptomatic OA (as defined below) at just one joint site were required to have structural abnormalities at least one other joint site, defined by the presence of ROA in any of the four joints, or the presence of two or more Heberden's nodes, Bouchard's nodes, or squaring of at least one CMC1 joint on physical examination. Symptomatic OA in the knee and hip was defined according to the American College of Rheumatology (ACR) recommendations for knee and hip OA[19;20]. Knee OA was defined as pain or stiffness for most days of the preceding month and osteophytes at the joint margins of the tibiofemoral joint (*x* ray spurs).

Hip OA was defined as pain or stiffness in the groin and hip region on most days of the preceding month in addition to femoral or acetabular osteophytes or axial joint space narrowing on radiography. Prosthetic joints in the hips or knees as a result of end stage OA were defined as OA in that particular joint. Spine OA (cervical and lumbar) was defined as pain or stiffness in the spine on most days of the preceding month, in addition to a Kellgren/Lawrence score of two in at least one disc or one apophyseal joint.

OA in hand joints was defined according to the ACR criteria[21] as pain or stiffness on most days of the preceding month in addition to three of the following four criteria: bony swelling in two or more of the ten selected joints (bilateral DIP joints 2+3, bilateral PIP joints 2+3, and CMC1 joints), bony swelling of in two or more DIP joints, fewer than three swollen MCP joints, and deformity of at least one of the ten selected joints.

Intrareader variability for the different joint sites, scored by the Kellgren/Lawrence method, was assessed as follows: the intraclass correlation coefficient (ICC, with 95% confidence interval) was for the hands, 0.95 (0.92 to 0.96); for the knees (tibiofemoral), 0.92 (0.86 to 0.96); for the hips, 0.95 (0.92 to 0.98); for the cervical spine (apophyseal and disc), 0.71 (0.52 to 0.84); and for the lumbar spine (apophyseal and disc), 0.67 (0.46 to 0.81). Intrareader variability was based on an examination of 40 radiographs that were selected randomly throughout the duration of the study period and were blinded for any patient characteristics.

To confirm previous findings, a subanalysis was carried out for OA in the different hand joints. DIP or CMC1 OA cases were defined by pain or stiffness at a CMC1 or a DIP joint in addition to a Kellgren/Lawrence score of at least two in a CMC1 or DIP joint. From all CMC1 or DIP cases in the GARP study, 86% and 75% respectively, met the Icelandic criteria for these phenotypes. In this analysis, sibling pairs of the GARP study were compared with the complete sample of the Rotterdam study as control group, which is called "the Rotterdam sample" and represents the normal population.

Statistical analysis

Differences in allele frequencies between case and control groups from the Rotterdam study were calculated by Pearson's chi-square test or Fisher's exact test for rare alleles. For the distribution of genotypes, the Hardy-Weinberg equilibrium (HWE) was tested by using the HWE program of LINKUTIL (http://linkage.rockefeller.edu/ott/linkutil.htm) or the exact HWE test for rare alleles implemented in R version 1.9.1, (http://www.r-project.org/). All genotyped SNPs were in HWE. A logistic regression model was fitted to measure the strength of association, which is expressed as odds ratios (OR) with 95% confidence intervals (95% CI) adjusted for age (years), body mass index (BMI, kg/m^2), and sex. In these analyses, homo- and heterozygous carriers of the risk allele were pooled.

For differences in allele or genotype frequencies between sibling pairs from the GARP study and the Rotterdam sample, standard errors were estimated from the variance between sibling pairs (robust standard errors)[22]. Instead of adjusting *P* values a priori (for example, for multiple testing), exact *P* values are provided in order to allow the reader to interpret the level of significance. We carried out robust standard error analyses using Stata SE8 software (Stata Corporation, USA). All other analyses were done with SPSS version 11 software (SPSS, Chicago, Illinois, USA).

A haplotype-based approach using tagging SNPs from The International HapMap project[23] was applied to examine whether a specific haplotype is underlying the observed association of the A allele of SNP6 with CMC1 OA in the GARP study. The HapMap (public data release 11) indicated eight tagging SNPs with an allele frequency ≥ 0.05 to capture haplotypes above 5%, encompassing three haplotype blocks, in the *MATN3* region (covering 100 kb). Initially, we genotyped four tagging SNPs (rs3769762, rs1191818, rs2244939 and rs3731663) in all subjects, to survey which tagging SNPs delineated the risk haplotype. Haplotype analysis revealed that the risk haplotype was delineated by rs1191818 and rs3731663.

In the Rotterdam sample, measures of linkage disequilibrium (LD) expressed as Lewontin's |D'| coefficient, and simultaneous estimation of haplotype frequencies and of their associated effects on the phenotype of interest were carried out using the Stochastic Expectation Maximization (EM) algorithm implemented in the THESIAS Program (THESIAS version 2, http://ecgene.net/genecanvas/modules/news/)[24]. In the Rotterdam sample, haplotype effects were calculated between previously described patient and control groups.

For calculating haplotype frequencies and effects in the GARP study, haplotype probabilities were estimated in the Rotterdam sample and probands or in the Rotterdam sample and siblings separately using the EM algorithm implemented in SNPHAP (SNPHAP version 1.3, http://www-gene.cimr.cam.ac.uk/clayton/software/). Haplotype frequencies in either probands or siblings or in all sibling pairs were estimated by weighting for posterior haplotype probability as calculated with SNPHAP. The strength of these effects in all sibling pairs as compared to the Rotterdam sample as control group was determined using logistic regression with robust standard errors to adjust for family relationship[22]. In both THESIAS and SNPHAP, we used the missing data option, which allows us to carry out haplotype analysis using subjects with some missing genotype measurements.

Genotype measurements

Genomic DNA was isolated from blood samples. In total, 809 subjects (331 men, 478 women) from the Rotterdam study and 191 sibling pairs (n = 382) were genotyped for three polymorphisms located in the *MATN3* gene denoted in NCBI dbSNP build 117 (http://www.ncbi.nlm.nih.gov/SNP/): rs2242190; SNP3, rs8176069; SNP5 and rs8176070; SNP6 (nomenclature as described by Stefansson et al.) and four tagging SNPs rs3769762, rs1191818, rs2244939 and rs3731663) indicated in the HapMap.

All PCR reactions contained 2.5 ng of genomic DNA and standard reagents. SNP5 and SNP6 were genotyped by restriction fragment length analysis using digestions of 5 µl PCR product with 0.1 µl AflII (New England Biolabs, Massachusetts, USA) or 0.1 µl PciI (New England Biolabs) in a final volume of 15 µl, respectively. Digestion products were electrophoresed through 1.5% agarose gels stained with ethidium bromide. Six subjects, that had the rare T allele of SNP5, were sequenced to confirm the genotype of SNP6 (TG or TA). The remaining polymorphisms were genotyped by mass spectrometry (homogeneous Mass ARRAY system; Sequenom Inc., San Diego, CA), using standard conditions. Genotypes were analysed by using Genotyper 3.0 software (Sequenom Inc).

Results

Characteristics of subjects from both the Rotterdam sample and the GARP study are shown in Table 1. In the Rotterdam study, each specific case group was compared to a specific control group, which comprises the complete Rotterdam sample, excluding that specific case group. Significant differences in age, BMI and sex, between case and control groups were observed (not shown).

Subjects from the GARP study were compared to the complete Rotterdam sample as control group (n = 809), which represents the normal population. Using a population-based cohort as control group may be conservative for detecting association. Both the Rotterdam sample and the GARP study are Caucasian subjects from the western areas of the Netherlands with a mean age of 60.3 years and may represent the same genetic background. Because the frequency of women was significantly higher in the GARP study as compared to the Rotterdam sample, stratification for women only was carried out for significant results. Although significant differences in age, BMI and sex, between case and control groups were observed (not shown), the absolute values of age, BMI and sex were similar. BMI, age and sex were added as covariables in all logistic regression analyses to account for these differences.

Table 1 Characteristics of subjects with ROA from the Rotterdam sample and sibling pairs with symptomatic OA from the GARP study

	The Rotterdam study, ROA							The GARP study, symptomatic OA[2]		
Characteristic	Total	Hip[1]	Knee[1]	DD[1]	Hand[1]	DIP[1]	CMC1[1]	Total	DIP	CMC1
No. (frequency)	809 (1)	71 (0.09)	143 (0.18)	176 (0.23)	172 (0.21)	161 (0.20)	91 (0.11)	382 (1)	167 (0.44)	104 (0.27)
Age, mean ± SD (years)	60.3 ± 2.7	60.8 ± 2.5	60.9 ± 2.5*	61.0 ± 2.6*	61.4 ± 2.4*	61.0 ± 2.5	61.5 ± 2.5*	60.3 ± 7.5	61.5 ± 7.5*	61.3 ±7.4*
BMI, mean ± SD (kg/m^2)	26.3 ± 3.6	26.5 ± 3.4	28.2 ± 4.1*	26.9 ± 3.6*	27.0 ± 4.1*	26.7 ± 4.0*	26.5 ± 3.6	27.0 ± 4.7*	26.9 ± 4.4*	27.2 ± 5.0*
No. of women (frequency)	478 (0.59)	29 (0.41)*	102 (0.71)*	101 (0.57)	131 (0.76)*	134 (0.83)*	61 (0.67)	312 (0.82)*	146 (0.87)*	91 (0.88)*

R(OA) = (radiographic) osteoarthritis; GARP = Genetics, osteoARthritis and Progression; BMI = body mass index; DD = spinal disc degeneration; DIP = distal interphalangeal joint; CMC1 = first carpometacarpal joint

[1] Subjects for whom no phenotypic data were available were excluded in the analysis.

[2] Sibling pairs from the GARP study versus the Rotterdam sample as control group.

* $P \leq 0.05$ versus controls (the complete Rotterdam sample excluding those specific case groups).

Association analysis of SNP3, SNP5 and SNP6 in the Rotterdam sample

In the Rotterdam sample, association analysis was carried out for SNP3, SNP5 and SNP6 (nomenclature as described by Stefansson et al.) in different case and control groups, as previously defined (Table 2). In view of previous observations[4], we had anticipated SNP5 to be associated with hand OA phenotypes. In the Rotterdam sample, no significant differences between case groups with hand ROA, CMC1 ROA or DIP ROA and their control groups were observed. The frequency of the T allele of this SNP5, however, was significantly increased in subjects with spinal DD at two or more levels (0.031, $P = 0.007$) compared with the controls (0.009). Carriers of this T allele, had an increased risk of 2.9 (95% CI, 1.2-7.3, $P = 0.02$), of having spinal DD at two or more levels. Further stratification for sex did not reveal any sex specific associations (data not shown).

We estimated haplotype frequencies and effects among subjects with spinal DD at two or more levels and their controls. In the whole Rotterdam sample, we observed five haplotypes, represented by SNP3, SNP5 and SNP6, respectively (Table 4). The frequencies of the A-T-G haplotype, containing the risk T allele of SNP5, showed identical frequencies to the T allele of SNP5 alone.

Table 2 Allele frequencies of three *MATN3* variants in the population-based Rotterdam sample

Phenotype	SNP3[1]	SNP5[1]	SNP6[1]
Total	0.39 (829/523)	0.014 (1,526/22)	0.21 (1,217/329)
No knee ROA	0.39 (673/437)	0.016 (1,244/20)	0.21 (1,000/268)
Knee ROA	0.35 (149/81)	0.004 (269/1)	0.22 (207/57)
No hip ROA	0.39 (743/467)	0.013 (1,370/18)	0.21 (1,089/297)
Hip ROA	0.41 (72/50)	0.022 (135/3)	0.20 (110/28)
No spinal DD	0.39 (627/397)	0.009 (1,165/11)	0.22 (917/251)
Spinal DD	0.39 (177/111)	0.031 (312/10)*	0.20 (262/66)
No hand ROA	0.38 (650/394)	0.015 (1,192/18)	0.21 (955/251)
Hand ROA	0.42 (174/126)	0.009 (327/3)	0.23 (256/76)
No DIP ROA	0.39 (653/415)	0.015 (1,216/18)	0.21 (970/262)
DIP ROA	0.38 (171/105)	0.010 (303/3)	0.21 (241/65)
No CMC1 ROA	0.38 (733/447)	0.014 (1,349/19)	0.21 (1,077/289)
CMC1 ROA	0.45 (91/73)	0.012 (170/2)	0.22 (134/38)

ROA = radiographic osteoarthritis; DD = disc degeneration; DIP = distal interphalangeal joint;
CMC1 = first carpometacarpal joint

[1] Frequencies of the minor alleles (number of common alleles/number of minor alleles); SNP3 (G>A), SNP5 (C>T), SNP6 (G>A).

* $P \leq 0.01$, versus controls (no DD).

Association analysis of SNP3, SNP5 and SNP6 in the GARP study

In the GARP study, we investigated the association of SNP3, SNP5 and SNP6 in relation to symptomatic hand OA phenotypes (hand OA, CMC1 OA and DIP OA) and to familial OA at multiple joint sites (Table 3). Because no significant differences were found between the case and control groups of the Rotterdam sample for these phenotypes, the total Rotterdam sample was compared to the cases of the GARP study. We did not observe a significant relationship of either SNP3 or SNP5 with any of the definitions of spinal DD (data not shown), OA in hand or OA at multiple joint sites. However, the A allele of SNP6 was significantly increased in subjects with OA of the CMC1 joint (0.29, $P = 0.01$), compared with the Rotterdam sample as the control group (0.21). Among carriers of one or more risk alleles, this A allele conferred an adjusted OR of 2.0 (95% CI, 1.3-3.1, $P = 0.004$) for CMC1 OA. Stratification for women only revealed that carriers of the A allele had a somewhat higher adjusted OR of 2.2 (95% CI, 1.4-3.7, $P = 0.001$). We carried out haplotype analysis of the three SNPs in these sibling pairs to explore whether the association of the common SNP6 is represented by a specific haplotype (Table 4). The common A-C-A haplotype (containing the A allele of SNP6) conferred a slightly increased risk of 1.5 (95% CI, 1.1-2.1, $P = 0.02$) in all sibling pairs with CMC1 OA compared with the Rotterdam sample as control group.

To further delineate this possible risk haplotype, we included two tagging SNPs in the haplotype analysis. These haplotypes were represented by the five polymorphisms in the following order: rs1191818 (T>C), SNP3 (G>A), SNP5 (C>T), SNP6 (G>A) and rs3731663 (C>T). Haplotype analysis of these five SNPs showed that the common C-A-C-A-T haplotype, encompassing the A-C-A haplotype, conferred a significant and increased risk of 1.7 (95 % CI, 1.1-2.7, $P = 0.02$) in all sibling pairs with CMC1 OA, and 2.1 (95% CI, 1.3-3.5, $P = 0.004$) in all sibling pairs with CMC1 OA, stratified for women only.

Table 3 Allele frequencies of three *MATN3* variants in sibling pairs with symptomatic OA (The GARP study)

Phenotype	SNP3[1]	SNP5[1]	SNP6[1]
The Rotterdam study			
Controls	0.39 (829/523)	0.014 (1,526/22)	0.21 (1,217/329)
The GARP study			
Total[2]	0.41 (445/307)	0.013 (750/10)	0.24 (583/179)
Hand OA	0.41 (321/223)	0.011 (542/6)	0.24 (418/132)
DIP OA	0.39 (198/128)	0.012 (326/4)	0.21 (262/70)
CMC1 OA	0.45 (112/92)	0.010 (204/2)	0.29 (148/60)*

GARP = Genetics, osteoARthritis and Progression; CMC1 = first carpometacarpal joint;
DIP = distal interphalangeal joint
[1] Frequencies of the minor alleles (number of common alleles/number of minor alleles); SNP3 (G>A), SNP5 (C>T), SNP6 (G>A).
[2] Sibling pairs with familial OA at multiple joint sites.
* $P \leq 0.01$ versus controls (the Rotterdam sample); P values and standard errors were adjusted for family relationship by using robust standard errors.

Table 4 Estimated haplotype frequencies of subjects from the Rotterdam sample and the GARP study

Haplotype[1]	The Rotterdam study, ROA			The GARP study, CMC1 OA		
	Controls[2] (*n* = 809)	No spinal DD (*n* = 620)	Spinal DD[3] (*n* = 163)	Total (*n* = 104)	Probands (*n* = 57)	Siblings (*n* = 47)
GCG	0.608	0.607	0.599	0.545	0.565	0.521
GCA	0.003	0.005	0.001	0.005	0.000	0.011
ACG	0.167	0.169	0.169	0.157	0.146	0.170
ACA	0.209	0.211	0.200	0.283§	0.280	0.287
ATG	0.014	0.009	0.031*	0.010	0.009	0.011

GARP = Genetics, osteoARthritis and Progression; (R)OA = (radiographic) osteoarthritis; CMC1 = first carpometacarpal joint; DD = disc degeneration

[1] Haplotype frequencies ≥ 0.01 in the following order: SNP3 (G>A), SNP5 (C>T), SNP6 (G>A) allowing missing genotype data.

[2] All subjects from the Rotterdam sample.

[3] Subjects for whom no phenotypic data were available were excluded in the analysis.

* $P \leq 0.01$, versus controls (no spinal DD subjects).

§ $P \leq 0.02$, versus controls.

Discussion

Our results indicate that SNP5 of the *MATN3* gene, previously associated with idiopathic hand OA in the Icelandic population[4], is involved in other heritable OA phenotypes, such as spinal DD. Carriers of this T allele revealed an increased risk of 2.9 (95% CI 1.2-7.3, $P = 0.02$) of having DD at two or more levels in the population-based Rotterdam sample. In the study by Stefansson et al.[4], no phenotypic data of spinal DD appeared to be available. We did not find any significant association of SNP5 with similar clinical hand OA, CMC1 OA or DIP OA phenotypes as described in the Icelandic study, or with radiographic hand phenotypes defined in the current study. Observations in the study by Stefansson et al.[4] suggested that the T allele of SNP5 might be important since the nucleotide change predicts a very conserved amino acid substitution[4]. We observed that the frequencies of the A-T-G haplotype are identical as the frequencies of the T allele of SNP5 alone.

Although a possible genetic relationship between generalised ROA and spinal DD was suggested, there is no consensus whether spinal DD constitutes a form of OA or is a distinct joint disease[16;25]. The lack of association of SNP5 with spinal DD in the GARP study may be due to the fact that only a few subjects in the GARP study carried the T allele of SNP5. Moreover, spinal DD in these selected sibling pairs might be part of a specific generalised OA phenotype.

Matrilins mediate interactions with cartilage oligomeric matrix protein, aggrecan, collagen type II and collagen type IX, presumably acting as adaptors connecting macromolecular networks[6;26-28]. Although the precise function of matrilin-3 is still unknown, variants in this gene may reduce its stabilising role in the extracellular cartilage matrix. Mutations in the region encoding the von Willebrand domain

(vWFA) of *MATN3* cause multiple epiphyseal dysplasia, mainly in the knee and hip joints[6-11]. In addition, a recent paper reported a homozygous mutation (C304S) located in the first epidermal growth factor-like domain, lying in the immediate vicinity of SNP5, resulting in a novel form of spondylo-epi-metaphyseal dysplasia[29].

Recently, Otten et al.[30] have attempted to elucidate the pathogenic mechanism of these matrilin-3 mutations at the cellular level. In this study, two point mutations causing chondrodysplasia (R121W and C304S) and SNP5 were introduced and the corresponding proteins were expressed in primary articular chondrocytes. In contrast to the chondrodysplasia mutations, SNP5 mutants were expressed, processed, secreted and incorporated in an extracellular network in a manner similar to the wild type matrilin-3[30]. These results and the small effect sizes in both the Icelandic and our study, suggest that SNP5 (T303M) results only in subtle structural and functional consequences or is not responsible for the observed effect.

As compared with the Icelandic study, we observed a two to three times higher allele frequency of the T allele of SNP5, especially in our control groups consisting of subjects from the population-based Rotterdam sample. This may be due to differences in genetic background. However, the allele frequencies of SNP3 and SNP6 are similar in our studies and the Icelandic study. The failure of replication of the previous findings in the Icelandic study may underlie in this frequency difference of SNP5 in the control group. Therefore, the allele frequency of SNP5 should be checked in other populations.

In combining the Rotterdam study and the GARP study, we have the opportunity to investigate the contribution of gene variants to the susceptibility of OA in four joint groups (hip, hand, knee and spinal DD). The Rotterdam study includes radiographic OA phenotypes irrespective of symptoms; the GARP study represents familial symptomatic OA at multiple sites. The lack of association of SNP5 with hand OA in our studies may be explained if this variant is associated specifically with symptomatic OA in the hands. We have limited information on symptomatic OA characteristics in the Rotterdam sample and we have a selection for OA at multiple joint sites in the GARP study. Consequently, we have a small number of SNP5 carriers in the GARP study.

In the GARP study, however, SNP6 of the *MATN3* gene indicated an association for OA at the CMC1 joint. Carriers of the A allele among the affected siblings had a significantly increased risk of 2.0 (95% CI 1.3-3.1 $P = 0.004$) of having CMC1 OA. An increased frequency of the A allele of SNP6 in subjects with CMC1 OA and the risk haplotype A-C-A for hand OA, was also observed in the study by Stefansson et al.; however, it appeared to be insignificant[4]. In the Rotterdam study, we did not have symptomatic CMC1 data available to replicate this association with SNP6. However, the function of SNP6 is unknown and it could be that SNP6 has no functional

consequences but is in LD with the causal variant. Our studies, therefore, did allow us to detect associations of SNP6 with symptomatic CMC1 OA and SNP5 with spinal DD, which emphasise that OA is heterogeneous and multifactorial in its aetiology. As in many genetic association studies, we cannot exclude the possibility of false positive findings due to multiple comparisons. At this stage we conclude that we did confirm previous results indicating the relevance for OA of the *MATN3* locus.

We observed a relatively high frequency of the A-C-A haplotype with a moderate risk for CMC1 OA. However, the three polymorphisms examined represent only a limited sample of the known haplotypes of the *MATN3* region. Therefore, we investigated whether a more extended haplotype using tagging SNPs delineated a more specific haplotype conferring a higher risk to CMC1 OA. Although these tagging SNPs as single polymorphisms showed no association with CMC1 OA, a common haplotype C-A-C-A-T was identified that conferred an increased significant risk of 1.7 (95 % CI, 1.1-2.7, $P = 0.02$) with CMC1 OA. For women, a higher significant risk of 2.1 (95% CI, 1.3-3.5, $P = 0.004$) with CMC1 OA was observed. However, an effect driven especially by female sex could not be determined owing to the small number of men in the GARP study. Furthermore, one other protective and rare haplotype was observed, which may imply that more relevant genetic variation of this locus is present which needs further investigation. Increasing the number of tagging SNPs could capture more relevant variation in the *MATN3* region, not necessarily at the *MATN3* gene itself.

Our results indicate that SNP5 of the *MATN3* gene, previously associated with idiopathic hand OA, is also involved in spinal DD. In addition, a common haplotype at this locus, other than that carrying the T allele of SNP5, was found to be a moderate risk factor increasing susceptibility of CMC1 OA. Together, our studies confirm the relevance of the *MATN3* locus to spinal disc degeneration and CMC1 OA. This needs replication in another OA study group.

Acknowledgements

We thank all participants of the Rotterdam and GARP study. The Rotterdam Study is supported by the Erasmus Medical Centre and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research, the Netherlands Organization for Health Research and Development, the Research Institute for Diseases in the Elderly, the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam.

The Leiden University Medical Centre, the Dutch Arthritis Association and Pfizer Inc., Groton, CT, USA support the GARP study. We further thank for the GARP study the support of the cooperating hospitals and referring rheumatologists, orthopaedic surgeons and general practitioners and for the Rotterdam study the support of general practitioners and pharmacists of the Ommoord district in Rotterdam. Finally, we thank Dennis Kremer and the Centre for Medical System Biology (CMSB) for their contribution to the genotyping of the SNPs.

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