

EXTENDED REPORT

Association of matrilin-3 polymorphisms with spinal disc degeneration and osteoarthritis of the first carpometacarpal joint of the hand

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Background: Seven polymorphisms in the *matrilin-3* (*MATN3*) gene were previously tested for genetic association with hand osteoarthritis in an Icelandic cohort. One of the variants, involving a conserved amino acid substitution (T303M; SNP5), was related to idiopathic hand osteoarthritis.

Objectives: To investigate SNP5 and two other promising polymorphisms (rs2242190; SNP3, rs8176070; SNP6) for association with radiographic and symptomatic hand osteoarthritis phenotypes, as well as other heritable phenotypes.

Methods: Polymorphisms were examined in two distinct cohorts of subjects: a population based sample from the Rotterdam study (n = 809), and affected siblings from the genetics, osteoarthritis and progression (GARP) study (n = 382).

Results: The originally described association of T303M with the hand osteoarthritis 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 to 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 to 3.1, p = 0.004) for osteoarthritis 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 osteoarthritis (odds ratio = 1.7 (95% CI, 1.1 to 2.7, p = 0.02)).

Conclusions: These associations suggest that the *MATN3* region also determines susceptibility to spinal disc degeneration and CMC1 osteoarthritis.

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Osteoarthritis is a common age related degenerative disease of the joints characterised by gradual loss of articular cartilage. Twin and sibling pair studies have shown that genetic factors play a substantial role in the aetiology of osteoarthritis. 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 variety of joint site or sex specific definitions of osteoarthritis, have been undertaken and have revealed positive linkage areas, some of which contain candidate genes for the susceptibility to osteoarthritis.^{2,3}

A scan of 329 Icelandic families with idiopathic hand osteoarthritis (involvement of either or both the distal interphalangeal (DIP) and first carpometacarpal (CMC1) joints) highlighted evidence for linkage on chromosome 2p, 4q, 3p.⁴ For the chromosome 2 loci, high LOD (log of odds) scores were reached for patients with affected CMC1 joints and for those with osteoarthritis in both CMC1 and DIP joints. Recently, the genome scan by Hunter *et al* revealed separate chromosomal regions for osteoarthritis of CMC1 and DIP joints, again by using a joint specific hand approach.⁵ Taken together, these studies indicate that osteoarthritis 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 non-collagenous extracellular oligomeric matrix protein.⁴ Mutations in this gene have previously been shown to cause different forms of multiple epiphyseal dysplasia.^{6–11} This disease is characterised by generalised dysplasia of epiphyses followed

by early onset osteoarthritis, 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 osteoarthritis, DIP osteoarthritis, or CMC1 osteoarthritis (0.01) as compared with the Icelandic population (0.005).⁴ In these patients, a moderately 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 osteoarthritis only or whether it is also involved in other heritable osteoarthritis phenotypes. In this report, we therefore investigated the findings of SNP5 in relation to hand osteoarthritis in a population based sample of the Rotterdam study and in an osteoarthritis affected sibling pair study (the Genetics, Osteoarthritis and Progression (GARP) study). 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 osteoarthritis phenotypes also at other joint sites in the

Abbreviations: ACR, American College of Rheumatology; CMC1, first carpometacarpal joint; DIP, distal interphalangeal joint; GARP, Genetics, Osteoarthritis and Progression study; htSNP, haplotype tag SNP; HWE, Hardy–Weinberg equilibrium; ROA, radiographic osteoarthritis; SNP, single nucleotide polymorphism

Rotterdam sample or the GARP study. Finally, we explored the initial associations of separate single nucleotide polymorphisms (SNPs) at this locus by haplotype analysis.

METHODS

The Rotterdam study

The Rotterdam study, which comprises 7983 white 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 School 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 osteoarthritis (ROA) of the knees, hips,¹⁵ the hands, and thoracolumbar 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 participants. After each set of about 150 radiographs the scores of the two readers were evaluated. Whenever the scores differed by 2 or more points, or was 2 for one reader but 1 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 trapezoscaphoid 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 ≥ 2 .¹⁷

In the Rotterdam sample, hand ROA at one or two hand joint groups (56% and 37%, respectively) or spinal disc degeneration at one level (61%) is already very prevalent and is likely to be a part of the normal wear and tear process. To enrich the sample for subjects who may be genetically predisposed to osteoarthritis, we divided the subjects into quartiles of the sum score 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 with subjects with hand ROA in fewer than three hand joint groups. Subjects affected by spinal disc degeneration at two or more disc levels (25% cut off value) were compared with 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 joint affected on each hand were compared with subjects with fewer than one DIP joint affected on each hand; subjects with CMC1 ROA in one or more CMC1 joints affected were compared with 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

The ongoing GARP study, which consists of white sibling pairs of Dutch origin affected predominantly by symptomatic osteoarthritis at multiple sites, is aimed at identifying determinants of osteoarthritis susceptibility and progression.¹⁸ Proband (ages 40–70 years) and their siblings had osteoarthritis at multiple joint sites of the hand or in ≥ 2 of the following joint sites (hand, spine (cervical or lumbar), knee or hip.¹⁸

Subjects with symptomatic osteoarthritis (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 ≥ 2 Heberden's nodes, Bouchard's nodes, or squaring of at least one CMC1 joint on physical examination. Symptomatic osteoarthritis in the knee and hip was defined according to the American College of Rheumatology (ACR) recommendations for knee and hip osteoarthritis.^{19, 20} Knee osteoarthritis 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 osteoarthritis 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 osteoarthritis were defined as osteoarthritis in that particular joint. Spine osteoarthritis (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 2 in at least one disc or one apophyseal joint.

Osteoarthritis in hand joints was defined according to the ACR criteria²¹ as pain or stiffness on most days of the preceding month, in addition to three of the following four criteria: bony swelling of two or more of the 10 selected joints (bilateral DIP joints 2+3, bilateral PIP joints 2+3, and CMC1 joints), bony swelling of two or more DIP joints, fewer than three swollen MCP joints, and deformity of at least one of the 10 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 replicate previous findings, a subanalysis was carried out for osteoarthritis in the different hand joints. DIP or CMC1 osteoarthritis cases were defined by pain or stiffness at a CMC1 or a DIP joint in addition to a Kellgren/Lawrence score of at least 2 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 χ^2 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

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

Characteristics	The Rotterdam sample (ROA)					The GARP study (symptomatic osteoarthritis)†				
	Total	Hip ROA*	Knee ROA*	DD*	Hand ROA*	DIP ROA*	CMC1 ROA*	Total	DIP OA	CMC1 OA
Number (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 (SD)	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 (SD)	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)‡
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)‡

*Subjects for whom no phenotypic data were available were excluded in the analysis.

†Subjects in the GARP study were compared with controls, the Rotterdam sample.

‡p ≤ 0.05 v controls (the complete Rotterdam sample excluding those specific case groups).

BMI, body mass index; CMC1, first carpometacarpal joint; DD, distal interphalangeal joint; GARP, Genetics, Osteoarthritis and Progression study; OA, osteoarthritis; ROA, radiographic osteoarthritis.

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 (CI) adjusted for age (years), body mass index (BMI, kg/m²), and sex. In these analyses, homozygous 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 haplotype tag SNPs (htSNPs) from The International HapMap project²³ was applied to examine whether a specific haplotype is underlying the observed association of the A allele of SNP6 with CMC1 osteoarthritis in the GARP study. The HapMap (public data release 11) indicated eight htSNPs 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 htSNPs (rs3769762, rs1191818, rs2244939, and rs3731663) in all subjects, to survey which htSNPs 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 Maximisation (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 SNP HAP (SNP HAP 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 SNP HAP. The strength of these effects in all sibling pairs as compared with 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 SNP HAP, 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 all, 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 htSNPs rs3769762, rs1191818, rs2244939, and rs3731663) indicated in the HapMap.

All polymerase chain reactions (PCR) contained 2.5 ng of genomic DNA and standard reagents. SNP5 and SNP6 were

Table 2 Allele frequencies of 3 MATN3 variants in the population based Rotterdam sample

Phenotype	SNP3*	SNP5*	SNP6*
Total	0.39 (829/523)	0.014 (1526/22)	0.21 (1217/329)
No knee ROA	0.39 (673/437)	0.016 (1244/20)	0.21 (1000/268)
Knee ROA	0.35 (149/81)	0.004 (269/1)	0.22 (207/57)
No hip ROA	0.39 (743/467)	0.013 (1370/18)	0.21 (1089/297)
Hip ROA	0.41 (72/50)	0.022 (135/3)	0.20 (110/28)
No DD	0.39 (627/397)	0.009 (1165/11)	0.22 (917/251)
DD	0.39 (177/111)	0.031 (312/10)†	0.20 (262/66)
No hand ROA	0.38 (650/394)	0.015 (1192/18)	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 (1216/18)	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 (1349/19)	0.21 (1077/289)
CMC1 ROA	0.45 (91/73)	0.012 (170/2)	0.22 (134/38)

*Frequencies of the minor alleles (n common allele/n minor allele); SNP3 (G>A), SNP5 (C>T), SNP6 (G>A).

†p ≤ 0.01 v controls (no disc degeneration).

CMC1, first carpometacarpal joint; DD, spinal disc degeneration; DIP, distal interphalangeal joint; ROA, radiographic osteoarthritis; SNP, single nucleotide polymorphism.

genotyped by restriction fragment length analysis using digestions of 5 µl PCR product with 0.1 µl AflIII (New England Biolabs, Beverly, 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 who 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, California, USA), 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 with 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 white 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 with 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 these variables were similar. BMI, age, and sex were added as covariables in all logistic regression analyses to account for these differences.

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 that SNP5 would be associated with hand osteoarthritis 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 disc degeneration 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 to 7.3; p = 0.02) of having spinal disc degeneration 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 disc degeneration at two or more levels and their controls. In the whole Rotterdam sample, we

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

Phenotype	SNP3*	SNP5*	SNP6*
<i>Rotterdam study</i>			
Controls	0.39 (829/523)	0.014 (1526/22)	0.21 (1217/329)
<i>GARP study</i>			
Total†	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)‡

*Frequencies of the minor alleles (n common allele/n minor allele); SNP3 (G>A), SNP5 (C>T), SNP6 (G>A).

†Subjects with familial osteoarthritis at multiple joint sites.

‡p ≤ 0.01 v controls (the Rotterdam sample); p values and standard errors were adjusted for family relationship by using robust standard errors.

CMC1, first carpometacarpal joint; DIP, distal interphalangeal joint; GARP, Genetics, Osteoarthritis and Progression study; OA, osteoarthritis; SNP, single nucleotide polymorphism.

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

Haplotype*	The Rotterdam study, ROA			The GARP study, CMC1 OA		
	Controls† (n = 809)	No DD (n = 620)	DD‡ (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

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

†All subjects from the Rotterdam sample.

‡Subjects for whom no phenotypic data were available were excluded in the analysis.

¶ $p \leq 0.01$ v controls (no disc degeneration subjects).

§ $p \leq 0.02$ v controls.

CMC1, first carpometacarpal joint; DD, spinal disc degeneration; DIP, distal interphalangeal joint; GARP, Genetics, Osteoarthritis and Progression study; OA, osteoarthritis; ROA, radiographic osteoarthritis.

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.

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 osteoarthritis phenotypes (hand osteoarthritis, CMC1 osteoarthritis, and DIP osteoarthritis) and to familial osteoarthritis 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 relation of either SNP3 or SNP5 with any of the definitions of spinal disc degeneration (data not shown), osteoarthritis in the hand, or osteoarthritis at multiple joint sites. However, the A allele of SNP6 was significantly increased in subjects with osteoarthritis 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 to 3.1; $p = 0.004$) for CMC1 osteoarthritis. Stratification for women only revealed that carriers of the A allele had a somewhat higher adjusted OR of 2.2 (95% CI, 1.4 to 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 to 2.1; $p = 0.02$) in all sibling pairs with CMC1 osteoarthritis compared with the Rotterdam sample as control group.

To further delineate this possible risk haplotype, we included two htSNPs 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 to 2.7; $p = 0.02$) in all sibling pairs with CMC1 osteoarthritis, and 2.1 (95% CI, 1.3 to 3.5; $p = 0.004$) in all sibling pairs with CMC1 osteoarthritis, stratified for women only.

DISCUSSION

Our results indicate that SNP5 of the *MATN3* gene, previously associated with idiopathic hand osteoarthritis in the Icelandic

population,⁴ is involved in other heritable osteoarthritis phenotypes such as spinal disc degeneration. Carriers of this T allele showed an increased risk of 2.9 (95% CI, 1.2 to 7.3, $p = 0.02$) of having disc degeneration at two or more levels in the population based Rotterdam sample. In the study by Stefansson *et al*,⁴ no phenotypic data on spinal disc degeneration appeared to be available. We did not find any significant association of SNP5 with similar clinical hand osteoarthritis, CMC1 osteoarthritis, or DIP osteoarthritis phenotypes as described in the Icelandic study, or with the 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 as the nucleotide change predicts a very conserved amino acid substitution.⁴ We observed that the frequencies of the A-T-G haplotype are identical to the frequencies of the T allele of SNP5 alone.

Although a possible genetic relation between generalised ROA and spinal disc degeneration was suggested, there is no consensus as to whether spinal disc degeneration constitutes a form of osteoarthritis or is a distinct joint disease.^{16, 25} The lack of association of SNP5 with spinal disc degeneration in the GARP study may be because only a few subjects in the GARP study carried the T allele of SNP5. Moreover, spinal disc degeneration in these selected sibling pairs might be part of a specific generalised osteoarthritis 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-epimetaphyseal 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 reflect 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 osteoarthritis in four joint groups (hip, hand, knee, and spinal disc degeneration). The Rotterdam study includes radiographic osteoarthritis phenotypes irrespective of symptoms; the GARP study represents familial symptomatic osteoarthritis at multiple sites. The lack of association of SNP5 with hand osteoarthritis in our studies may be explained if this variant is associated specifically with symptomatic osteoarthritis in the hands. We have limited information on symptomatic osteoarthritis characteristics in the Rotterdam sample and we have a selection for osteoarthritis 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 osteoarthritis 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 to 3.1; $p = 0.004$) of having CMC1 osteoarthritis. An increased frequency of the A allele of SNP6 in subjects with CMC1 osteoarthritis and the risk haplotype A-C-A for hand osteoarthritis was also observed in the study by Stefansson *et al*; however, it appeared to be non-significant.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 linkage disequilibrium with the causal variant.

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

We observed a relatively high frequency of the A-C-A haplotype with a moderate risk for CMC1 osteoarthritis. 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 htSNPs delineated a more specific haplotype conferring a higher risk of CMC1 osteoarthritis. Although these htSNPs as single polymorphisms showed no association with CMC1 osteoarthritis, a common haplotype C-A-C-A-T was identified that conferred an increased risk of 1.7 (95% CI, 1.1 to 2.7; $p = 0.02$) for CMC1 osteoarthritis. For women, a higher risk of 2.1 (95% CI, 1.3 to 3.5; $p = 0.004$) for CMC1 osteoarthritis 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 htSNPs 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 osteoarthritis, is also involved in spinal disc degeneration. 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 osteoarthritis. Together, our studies confirm the relevance of the *MATN3* locus for spinal disc degeneration and CMC1 osteoarthritis. This needs replication in another osteoarthritis study group.

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