

Radiographic Progression of Lumbar Spine Disc Degeneration Is Influenced by Variation at Inflammatory Genes

A Candidate SNP Association Study in the Chingford Cohort

Ana M. Valdes, PhD, Geraldine Hassett, MBBS, Deborah J. Hart, PhD,
and Tim D. Spector, MD

Study Design. A candidate gene association study in a longitudinal cohort.

Objective. To investigate the association between polymorphisms at 25 candidate genes and progression of individual radiographic features of lumbar disc degeneration (LDD).

Summary of Background Data. LDD is characterized radiographically by the presence of osteophytes and disc space narrowing and is known to have a genetic component. Because of the high prevalence of radiographic features, progression may be a more useful phenotype clinically to study than prevalence itself.

Methods. We tested the effect on radiographic progression of LDD of polymorphisms in 25 genes, 24 of which had been previously tested with regards to knee osteoarthritis. The progression traits used were the change in radiographic grade over 9 years in osteophytes, disc space narrowing, and summary Kellgren-Lawrence grade. Lumbar spine radiographs (L1–L5) at baseline and at follow-up were read for 720 women genotyped at the 25 genes participating in the Chingford study.

Results. Polymorphisms in MMP3, TIMP1, and COX2, which encode molecules involved in inflammatory pathways, were associated with radiographic progression of LDD. The strongest associations observed (statistically significant after correcting for multiple comparisons) were between COX2 and change in osteophyte grade ($P < 0.001$) and Kellgren-Lawrence grade ($P < 2 \times 10^{-5}$), and between the genes for vitamin D receptor ($P < 0.002$) and a thrombospondin (THSD2) ($P < 0.002$) and change in osteophyte grade.

Conclusions. Our results suggest a role for genes regulating inflammatory pathways in the radiographic progression of spine degeneration. This could prove a fruitful area for future therapeutics for the spine and other joints.

Key words: lumbar disc degeneration, genetic association, inflammatory genes, vitamin D receptor, single nucleotide polymorphisms. **Spine 2005;30:000–000**

Degenerative disc disease is a highly prevalent musculoskeletal disorder and a major cause of back symptoms.^{1,2} Its individual radiographic features affect the majority of the population as shown by recent epidemiologic studies.³

Spinal degeneration includes both osteoarthritic changes of the facet joint as well as disc degeneration.^{4–6} Facet joints are true synovial articulations that undergo degenerative changes identical to those of osteoarthritis (OA) seen in other synovial joints,⁷ namely, swelling, stiffness, deformity, instability, and decreased range of motion of the joint.⁸ Lumbar disc disease (LDD) is positively associated with OA of the facet joints⁹ and studies using magnetic resonance imaging (MRI) have shown that cartilage degeneration, especially thinning of the cartilage allows abnormal motion of the facet joint.⁴

Reports in the literature have also shown that lower back pain,¹⁰ sedentary occupation,¹¹ and lack of sports activity¹² are significant predictors for the development or deterioration of LDD. In addition, LDD has been shown to have a familial component^{13,14} and in some studies to be influenced by specific genetic risk factors such as COL9A1,^{15,16} aggrecan,¹⁷ vitamin D receptor (VDR),^{15,18–20} and matrix metalloprotease-3.¹⁵

Hassett *et al*²¹ reported that the presence of knee OA is a modest predictor of risk for progression of spine OA. Thus, it is possible that genetic risk factors for LDD partially overlap knee OA. Although the prevalence of mild radiographic OA features in the spine can be very high, not all individuals progress toward severe LDD. Radiographic evidence of degenerative change has been shown to be associated with increased age.²² Disc abnormalities have been reported to be extremely common among asymptomatic individuals.^{23–25} Thus, elucidating genetic factors that affect progression can help us understand the etiology of clinically relevant radiographic features of spine OA.

We have previously analyzed the role of polymorphisms at 24 knee OA candidate genes and reported several associations between these genetic polymorphisms and susceptibility and progression of knee OA.²⁶ In this study, we have tested the same polymorphisms of gene previously tested in association to radiographic knee OA plus a novel thrombospondin gene (THSD2)²⁷ with regards to progression of spine OA. We hypothesized that some of the same genetic factors affecting knee OA susceptibility would also influence the progression of spine OA.

From the Twin Research & Genetic Epidemiology Unit, St. Thomas Hospital, London, UK.

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Address correspondence and reprint requests to Tim D. Spector, MD, Twin Research & Genetic Epidemiology Unit, St. Thomas Hospital, London, SE1 7EH, UK; E-mail: tim.spector@kcl.ac.uk

■ Materials and Methods

Subjects. The Chingford Study population, established in 1988, is a well-described prospective longitudinal cohort of 1,003 women seen annually and described in detail previously.^{28–30} After a 9-year follow-up, 821 women remained for examination and DNA and paired radiographs were available for 720 women. All the women lived within 5 miles of the general practice, and 100% of those whose genotypes were analyzed were white. Women from this practice are similar to women in the U.K. general population in terms of weight, height, and smoking characteristics.³¹ Each woman was asked to undergo a radiographic examination of hands, knees, hips, and thoracolumbar spine. All women were given a nurse-administered standardized questionnaire. The standardized questionnaires asked details on medical history, drug treatment, joint symptoms, and the Medical Research Council back pain questionnaire to determine maximum number of days of pain in last month, year and duration, episodes of pain, and distribution. Occupation was current job, which was categorized into quartiles ranging from sedentary to heavy duty tasks depending on amount of activity (sitting, lifting, walking, bending) in occupation. A high proportion of the women were housewives, and these were coded also on previous occupation. Height was recorded in meters and weight in kilograms following physical examination. Body mass index (BMI) was calculated as weight (kg)/height (m²). Details of concomitant diseases, operations, or medications were also recorded. Sports data based on amount of sporting activity (not walking) in 1 week was divided into quartiles ranging from <0.5 hour a week of light sport to 2+ hours a week of vigorous activity sufficient to raise a sweat.³² Blood samples were drawn stored in EDTA and DNA extracted using standard phenol and salt methods. The St. Thomas Hospital and Waltham Forest Trust ethics committees approved the study protocol both for the original study and the 9-year follow-up. After study procedures were explained to participants, they gave written consent.

Radiographic Assessment. Lateral lumbar spine radiographs at years 1 and 9 were taken centered on the L3 vertebrae with the subjects in the left lateral recumbent position by the same radiographer at both time points. A single trained observer (G.H.) blinded to patient identity and chronologic order read all radiographs. Each lateral lumbar spine radiograph was graded 0 to 3 for the individual features of disc space narrowing (DSN) and for osteophyte (both anterior and posterior) formation grade and Kellgren-Lawrence (K/L) grade using the semiquantitative method reported by Lane *et al.*³³ Briefly, the grading system is as follows: Grade 0 = normal; Grade 1 = doubtful narrowing of joint space and possible osteophytic lipping; Grade 2 = definite osteophytes and possible narrowing of joint space; Grade 3 = moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour; Grade 4 = large osteophytes, marked narrowing of joint space, severe sclerosis, and definite deformity of bone contour. Within-observer variation was assessed by test-retest analysis of 40 randomly selected radiographs from the study. Good within-observer reproducibility ($\kappa = 0.78–0.89$) was found.

SNP Assays. Except for THSD2, the flanking sequences and the important information for all the SNPs in this study have been previously published.²⁶ The SNP in THSD2 falls 73 bp 3'

from the end of exon 3 of the THSD2 gene with flanking sequences: TGGAGGTGCTTTTCAAAGTCCTTTG [C/T] CAAAAGAAGTTGAAGGTTCTTGGGC. The allele frequencies at this SNP were 46% for the C allele and 54% for the T allele. No deviations from Hardy-Weinberg equilibrium were found for the genotype frequencies at this polymorphism.

Genotyping. Genomic DNA was set on 384 well plates. Genotyping was carried out using Assays-on-Demand SNP Genotyping products from Applied Biosystems (Applied Biosystems, Foster City, CA), where the primers are labeled with a reporter dye at the 5' end of each probe: the VIC dye linked to the 5' end of one of the allele's probe and the FAM dye was linked to the 5' end of the other allele's probe. TaqMan reactions were carried out using reagents supplied by the vendor (Applied Biosystems), with 900 nmol/L each of forward and reverse PCR primers and 200 nmol/L each of the FAM- and VIC-labeled TaqMan probes. Approximately 2 to 20 ng of genomic DNA was used per 15 μ L reaction. PCR amplification was carried out for 40 cycles under standard TaqMan conditions (UNG activation: 50 C, 2 minutes; AmpliTaq Gold activation: 95 C, 10 minutes; Denature: 95 C, 15 seconds; Anneal/extend: 60 C, 1 minute), using the ABI7900 Sequence Detection System in the 384 well format.

Statistical Methods. To assess progression of spine OA, the sum radiographic grade for L1–L2, L2–L3, L3–L4, L4–L5 for osteophytes (sum of anterior and posterior), K/L, and DSN were first computed. Some epidemiologic studies suggest that severity of radiographic features, rather than presence/absence of a feature, may be more closely associated with back pain and symptoms of LDD.³ To take into account both severity and progression at year 9 in proportion to baseline status, we used the following formula: Change in grade = (Sum of grade L1–L5 year 9 – Sum L1–L5 year 1)/Sum L1–L5 year 1.

Genetic associations. Analyses of variance were used to compare the mean radiographic grade progression between SNP genotypes. Age and BMI were used as covariates in the analysis of variance. The adjusted means of the change radiographic grade of each trait with standard error are shown.

Multiple comparisons and permutation test. To correct for the effects of multiple testing, we used a resampling permutation method. Permutation methods are well established as a robust approach for obtaining overall significance levels while minimizing Type II error.^{24–36} The permutation tests rely on the assumption that the LDD progression related phenotypes of an individual are fixed, and the null hypothesis (*i.e.*, no genetic association) is that the genotypes at the loci studied have no effect on the clinical traits. Under the null hypothesis of no genetic association, the genotypes of each individual should be exchangeable. We randomly shuffled the observed clinical values (keeping the three phenotypic traits together to preserve the correlation between them) over the 27 SNP genotypes of the 25 genes studied (keeping the 27 genotypes of each individual together) and computed the test statistics in these new samples. The test statistics used were a χ^2 for binary traits comparing genotype frequencies and an *F* value from the analysis of variance for the quantitative traits (change in K/L, osteophyte, and DSN grades). This procedure was repeated 500 times, generating an empirical distribution of the test under the hypothesis of no association between clinical traits and SNP genotypes. The *P* value corresponding to each of these tests statistics given the

number of degrees of freedom was then computed and the probability of observing by chance the total number of *P* values under 0.05 observed in the true set was estimated. To correct for individual *P* values, a false-discovery rate correction³⁷ was used. All statistical analyses were performed using the S-Plus package 2000 release 3.

Results

T1 The descriptive statistics of the individuals genotyped are shown in Table 1. The prevalence of the individual radiographic features studied was very similar to that reported among women of a similar age from Aberdeen.³ The presence of DSN (Grade 2 or higher) at one or more sites was 25.4% among the women from Aberdeen aged 65 years on average, and 26.5% in this study. The presence of osteophytes (Grade 2 or higher) was 33.8% in Aberdeen and 31.2% in this study at the 9-year follow-up.

The median change in the grade of individual radiographic features (IRFs) depending on the study participant's level of physical activity, back pain, and knee OA are also shown (Table 1). The results are consistent with published reports, which indicate that lack of sports activity, sedentary occupation, and back pain are important predictors of LDD progression¹⁰⁻¹² and that the presence of knee OA is a modest predictor of progression of some radiographic features.²¹

The progression traits, adjusted for baseline and severity, were only modestly correlated with each other (*R*² = 0.04 both for change in K/L grade and change in DSN grade and for change in osteophyte grade and change in DSN grade, *R*² = 0.16 between change in osteophyte grade and change in KL grade), although

highly statistically significant with *P* < 1 × 10⁻⁶ in all cases.

T2 The three progression traits studied were found to be associated with several of the SNPs genotyped (Table 2). In particular, for the change in KL grade, we observed significant associations with SNPs in genes COMP, COX2, MMP3, THSD2, and VDR. For change in osteophyte grade, the genes associated were CD36, COX2, MMP3, OGN, and TIMP1, whereas for change in DSN grade only SOD3 and CD36 were significantly associated (Table 3).

T3 We did find that some of the genes affecting knee OA also affected spine radiographic traits, namely, CD36, COX2, and to a lesser extent, NCOR2 (affecting joint space narrowing in the knee), TNA (which affected K/L change in the knee), and ESR1 (affecting osteophytes in the knee). However, ADAM12, the gene showing the strongest association with knee OA in our previous study, was not associated with any of the spine traits studied. The other genes tested affecting knee OA but not lumbar spine disc degeneration are BMP2, OPG, and CILP.

Applying a false-discovery rate correction for multiple comparisons, we find that the nominal *P* values under 0.002 have a corrected *P* < 0.05, and the one nominal *P* < 2 × 10⁻⁵ has a corrected *P* < 0.0017. Thus, after correcting for multiple comparisons, at least four of the associations remain statistically significant. Moreover, overall we observed 11 instances with nominal *P* values < 0.05. By permutation analysis, we found that the mean number of *P* values expected is 3.91 (median = 3, SD =

Table 1. Descriptive Statistics: Prevalence of Individual Radiographic Features at Baseline and at Follow-up and Mean, Standard Error, Median, and Interquartile Ranges of Change in Radiographic Features Over Time

| Trait | Baseline | | 9-Year Follow-up | | | |
|--------------------------------------|------------------------------|-------------------|----------------------|-------------------|-----------------------|-------------------|
| Age (yr) [mean (SE)] | 53.7 (0.22) | | 62.7 (0.12) | | | |
| BMI (kg/m ²) [mean (SE)] | 25.5 (0.15) | | 26.9 (0.18) | | | |
| % study participants (n = 720) with: | | | | | | |
| K/L ≥ 2 at 1 or more sites* | 55.50 | | 76.10 | | | |
| DSN ≥ 2 at 1 or more sites* | 11.90 | | 26.50 | | | |
| Trait | Change in Osteophyte Grade†‡ | | Change in KL Grade†‡ | | Change in DSN Grade†‡ | |
| | Mean (SE) | Median (Q1, Q3) | Mean (SE) | Median (Q1, Q3) | Mean (SE) | Median (Q1, Q3) |
| Job activity | | | | | | |
| Bottom quartile | 0.41 (0.10) | 0.25 (0.00, 0.67) | 0.65 (0.10) | 0.39 (0.00, 1.00) | 0.62 (0.17) | 0.33 (0.00, 1.00) |
| Top quartile | 0.36 (0.06) | 0.17 (0.00, 0.54) | 0.67 (0.15) | 0.37 (0.08, 0.80) | 0.65 (0.09) | 0.33 (0.00, 1.00) |
| Sports activity | | | | | | |
| Bottom quartile (<0.5 hours/wk) | 0.40 (0.03) | 0.25 (0.00, 0.67) | 0.62 (0.04) | 0.35 (0.00, 1.00) | 0.66 (0.05) | 0.33 (0.00, 1.00) |
| Top quartile (2+ hours/wk) | 0.38 (0.08) | 0.25 (0.00, 0.67) | 0.74 (0.16) | 0.43 (0.00, 1.00) | 0.61 (0.11) | 0.25 (0.00, 1.00) |
| Back pain 9 years | | | | | | |
| Present | 0.40 (0.03) | 0.25 (0.00, 0.67) | 0.66 (0.04) | 0.39 (0.07, 1.00) | 0.64 (0.07) | 0.34 (0.00, 1.00) |
| Absent | 0.38 (0.04) | 0.20 (0.00, 0.67) | 0.64 (0.06) | 0.36 (0.00, 1.00) | 0.62 (0.05) | 0.20 (0.00, 1.00) |
| Knee OA (KL = 2+) | | | | | | |
| Present | 0.39 (0.04) | 0.25 (0.00, 0.70) | 0.61 (0.05) | 0.33 (0.00, 1.00) | 0.68 (0.06) | 0.34 (0.00, 1.00) |
| Absent | 0.36 (0.03) | 0.17 (0.00, 0.67) | 0.58 (0.04) | 0.34 (0.00, 1.00) | 0.57 (0.04) | 0.33 (0.00, 1.00) |

*L1-L2, L2-L3, L3-L4, L4-L5.

†Change in grade = (sum L1-L5 of grade year 9 - sum L1-L5 baseline)/sum L1-L5 baseline.

‡Sum of anterior and posterior osteophytes.

Table 2. SNPs Tested in This Study and Their Genetic Association With Three Spine OA Progression Traits

| Gene | SNP Position | SNP Alias | P< | | |
|---------|---------------------|--------------|----------------------------|--------------------|---------------------|
| | | | Change in Osteophyte Grade | Change in KL Grade | Change in DSN Grade |
| AACT | Ala(G)9Thr(A) | AACT_9 | 0.385 | 0.599 | 0.656 |
| | Intron 1 C > G | AACT_int | 0.948 | 0.450 | 0.579 |
| ACLP | Intron 18 C > T | ACLP_int | 0.132 | 0.847 | 0.610 |
| ADAM12 | Gly(G)48Arg(C) | ADAM12_48 | 0.710 | 0.688 | 0.769 |
| ADLICAN | Gly(C)2663Asp(T) | ADLICAN_2663 | 0.295 | 0.419 | 0.312 |
| BGN | Intron 7 G > T | BGN_int | 0.213 | 0.219 | 0.180 |
| BMP2 | Ser(T)87Ser(C) | BMP2_87 | 0.362 | 0.148 | 0.583 |
| | Ser(T)190Arg(A) | BMP2_190 | 0.518 | 0.210 | 0.427 |
| BMPR1A | Intron 10 Del > Ins | BMPR_int | 0.961 | 0.602 | 0.521 |
| CD36 | 5' UTR A > C | CD36_5p | 0.040* | 0.141 | 0.015* |
| CILP | Thr(C)395Ile(T) | CILP_395 | 0.298 | 0.295 | 0.305 |
| COMP | Asn(A)386Asp(G) | COMP_386 | 0.579 | 0.050* | 0.884 |
| COX2 | Val(G)102Val(C) | COX2_102 | 0.001* | 2.E-05* | 0.964 |
| CTSL | Intron 1 C > T | CTSL_int | 0.428 | 0.619 | 0.680 |
| DAF | Intron 7 A > G | DAF_int | 0.070 | 0.856 | 0.838 |
| ESR1 | Intron 1 T > C | ESR1_int | 0.635 | 0.125 | 0.070 |
| IBSP | Gly(A)195Glu(G) | IBSP_195 | 0.910 | 0.687 | 0.947 |
| MMP3 | Intron 4 C > T | MMP3_int4 | 0.033* | 0.011* | 0.127 |
| NCOR2 | Thr(A)1699Ala(G) | NCOR2_1699 | 0.472 | 0.365 | 0.064 |
| OGN | 3' UTR A > G | OGN_3p | 0.007* | 0.151 | 0.565 |
| OPG | 5' UTR C > T | OPG_5p | 0.963 | 0.805 | 0.553 |
| SOD3 | 3' UTR C > T | SOD3_3p | 0.246 | 0.940 | 0.016* |
| THSD2 | Intron 3 C > T | THSD2_int3 | 0.161 | 0.002* | 0.287 |
| TIMP1 | Leu(C)124Leu(T) | TIMP1_124 | 0.006* | 0.663 | 0.707 |
| TNA | Ser(A)106Gly(G) | TNA_106 | 0.258 | 0.091 | 0.147 |
| TNFAIP6 | Arg(G)144Gln(A) | TSG_144 | 0.377 | 0.650 | 0.611 |
| VDR | Ile(T)365Ile(C) | VDR_365 | 0.254 | 0.002* | 0.274 |

*P < 0.05.

2.05) and that this number of significant associations was observed only in 1 of 500 permutations corresponding to an overall empirical probability of 0.002 of finding a pattern as extreme or more than the actual one observed. Therefore, we conclude that the strongest of the genetic associations are likely to be real and not due purely to the large number of comparisons carried out. The mean change in grade at each trait for the genotypes significantly associated is shown in Table 3. The largest differences in mean change in radiographic grade between genotypes coincide with the smallest P values.

Finally, we evaluated to what extent the SNPs were associated with progression of IRFs also associated with self-reported back pain at baseline and at the 9-year follow-up. Only the SNP at MMP3 was significantly associated with the presence of back pain at baseline with an odds ratio of 1.63 (95% confidence interval [CI] = 1.11, 2.39; P < 0.02). At the 9-year follow-up, none of the SNPs was significantly associated with pain, although the SNPs at COX2 and MMP3 showed an increased odds ratio of 2.44 (95% CI = 0.91, 6.51; P < 0.07) and 1.40 (95% CI = 0.95, 2.08; P < 0.09), respectively.

Discussion

Our findings confirm that several genes influence LDD, as previous studies had indicated (for example, Noponen-Hietala *et al*¹⁵). These data also suggest that inflammatory mediators, the thrombospondin family, and VDR are all important.

There are several limitations to the study that need mentioning. The findings of this study apply only to middle-aged women and may not hold for other populations. Along with the possibility of observing false positives, we must consider false negatives. For most genes, only one polymorphism has been studied; thus, it is feasible that genetic variation at these genes could still be involved in progression of LDD; but by not studying every variant at each gene and the resulting haplotype combinations, we might have missed such effects. Therefore, our inability to detect a genetic association at any given gene does not preclude a potentially important role for that gene in the radiographic progression of LDD.

Radiographs are not the gold standard for disc disease and ideally MRI should be used. Limitations of radiographic assessment include no assessment of changes in the endplate and vertebral body and insufficient categories to distinguish the wide range of appearances encountered. However, no longitudinal MRI population data exist with similar numbers or a 9-year period, and our radiograph data are still relatively unique. We did not have true quantitative data as scoring systems are semi-continuous and these usually fit standard models less well than fully continuous data. However, any errors of classification would tend to be random, as the scoring was blind to genotyping and therefore favors a null result.

Confounders are also important to consider; however, BMI, the most important for knee and hip OA, was not a significant factor in LDD progression in this data-

Table 3. Magnitude and Direction of Genetic Associations With Spine OA Progression Traits

| SNP Alias | Genotype | Change in KL Grade* | Standard Error | <i>F</i> (<i>df</i>) | <i>P</i> < |
|------------|----------|---------------------|----------------|--------------------------|--------------------|
| COMP_386 | AG | 0.841 | 0.109 | <i>F</i> (1,685) = 3.84 | 0.050 |
| | AA | 0.615 | 0.037 | | |
| MMP3_int4 | GG | 0.809 | 0.075 | <i>F</i> (1,684) = 6.42 | 0.011 |
| | GT+TT | 0.592 | 0.039 | | |
| COX2_102 | CC | 1.529 | 0.210 | <i>F</i> (1,679) = 18.32 | 2×10^{-5} |
| | CG+GG | 0.618 | 0.036 | | |
| THDS2_int3 | CC+CT | 0.708 | 0.042 | <i>F</i> (1,654) = 18.32 | 0.002 |
| | TT | 0.460 | 0.068 | | |
| TNA_106 | AA | 0.559 | 0.085 | <i>F</i> (1,683) = 2.89 | 0.091 |
| | AG | 0.591 | 0.057 | | |
| VDR_365 | GG | 0.705 | 0.053 | <i>F</i> (1,687) = 9.65 | 0.002 |
| | TT+TC | 0.589 | 0.038 | | |
| | CC | 0.875 | 0.083 | | |

| SNP Alias | Genotype | Change in Osteophyte Grade* | Standard Error | <i>F</i> (<i>df</i>) | <i>P</i> < |
|-----------|----------|-----------------------------|----------------|-------------------------|------------|
| CD36_5p | AA | 0.481 | 0.054 | <i>F</i> (1,692) = 4.22 | 0.040 |
| | AC+CC | 0.358 | 0.026 | | |
| DAF_int | AA | 0.344 | 0.036 | <i>F</i> (1,682) = 3.29 | 0.070 |
| | AG | 0.393 | 0.035 | | |
| MMP3_int4 | GG | 0.501 | 0.078 | <i>F</i> (1,684) = 4.54 | 0.033 |
| | GT+TT | 0.476 | 0.051 | | |
| COX2_102 | CC | 0.354 | 0.027 | <i>F</i> (1,679) = 6.13 | 0.001 |
| | CG+GG | 0.831 | 0.141 | | |
| OGN_3p | AA | 0.369 | 0.024 | <i>F</i> (1,667) = 7.24 | 0.007 |
| | AG | 0.358 | 0.025 | | |
| TIMP1_124 | AA+AG | 0.579 | 0.078 | <i>F</i> (1,684) = 7.73 | 0.006 |
| | GG | 0.343 | 0.027 | | |
| | GG | 0.494 | 0.046 | | |

| SNP Alias | Genotype | Change in DSN Grade* | Standard Error | <i>F</i> (<i>df</i>) | <i>P</i> < |
|------------|----------|----------------------|----------------|-------------------------|------------|
| CD36_5p | AA | 0.824 | 0.090 | <i>F</i> (2,692) = 5.91 | 0.015 |
| | AC+CC | 0.582 | 0.043 | | |
| NCOR2_1699 | GG | 0.408 | 0.183 | <i>F</i> (1,682) = 3.43 | 0.065 |
| | GA | 0.549 | 0.081 | | |
| ESR_int | AA | 0.676 | 0.047 | <i>F</i> (1,693) = 3.30 | 0.069 |
| | TT | 0.741 | 0.075 | | |
| SOD3_3p | TC+CC | 0.581 | 0.045 | <i>F</i> (1,669) = 5.79 | 0.016 |
| | CC | 0.516 | 0.059 | | |
| | CT+TT | 0.707 | 0.052 | | |

*All means are adjusted for age and BMI.

set. Age is another potential factor that strongly affects osteophyte and DSN progression in this set. However, the effect of age is similar to that of some of the SNPs, for example, the mean change in osteophyte grade of individuals under the age of 60 at baseline was 0.35 (standard error ± 0.02), whereas for those aged 60 and over was 0.48 (standard error ± 0.04). Such a difference is not larger than the one observed between genotypes at MMP3, COX2, OGN, TIMP1, and CD36, suggesting that, at least using this measure for progression, the contribution of genetic factors to the progression of LDD individual radiographic features can be as large as that of age. Finally, sports and occupation did not affect the genetic associations observed (not shown).

For the same radiographic features, other measures of progression can be envisioned that do not adjust for severity (grade at follow-up) or for baseline grade or that focus only on the worst affected joint at either baseline or follow-up. We have not tested these potential measures

of progression, and it is conceivable that some results could vary somewhat using different measures.

Having considered the above limitations, we found there was only partial overlap between genes involved in knee OA and in spine IRF progression. Two genes not associated with knee OA but associated with progression of LDD were SOD3, encoding an extracellular superoxide dismutase, and OGN, encoding osteoglycin, a small proteoglycan that induces ectopic bone formation.

The two thrombospondin genes tested, THSD2 and COMP, were associated with change in K/L grade. The thrombospondin receptor CD36 was, on the other hand, associated with both change in osteophyte and DSN grade. The thrombospondins are a family of extracellular proteins, some of which bind specific types of collagen, that participate in cell-to-cell and cell-to-matrix communication regulating cellular phenotype during tissue genesis and repair.³⁸ Our results suggest that this

class of molecules might be important in the pathogenesis of LDD and thus could represent a potential intervention target.

We also have confirmed a role for genetic variation at the VDR, which had been previously reported as being important cross-sectionally by several authors using different but closely related polymorphisms.^{15,18–20} The small *P* value and the fact that this gene has been associated with LDD in the past add weight and validity to our other findings.

In addition to COX2, two other genes in inflammatory pathways, MMP3 and TIMP1, were also associated with progression of LDD (Table 3). The results with MMP3 are also consistent with a known role for matrix metalloproteases in disc degradation^{39,40} and with the published genetic association with MMP3.¹⁵ It is also interesting to note that the strong association of these inflammatory SNPs with progression of IRFs is not reflecting strong correlation of the same SNPs with back pain, suggesting different mechanisms. The strongest and most striking association was with the COX2 SNP. Prostaglandin E2 (PGE2) is one of the most important mediators contributing to pathogenetic components of lumbar disc herniation and cyclooxygenase-2 (COX2) is the rate-limiting enzyme of PGE2 synthesis. The expression pattern in herniated discs indicate that COX-2 and inflammatory cytokines such as IL-8 are involved in lumbar disc herniation through the up-regulation of PGE2 synthesis.^{41,42} The large effect of genetic variation at COX2 on the progression of LDD is then not necessarily surprising. However, it could suggest that some individuals with particular genetic predisposition might benefit the most from early intervention in the form of cyclooxygenase-2 inhibitor pharmacotherapy.

Conclusion

We have reported a number of genes associated with progression of lumbar disc degeneration, some of which overlap with those affecting knee osteoarthritis and involve the thrombospondin family and inflammatory mediators. As for any association study, replication in a different study sample should be the next step to confirm the role and importance of the genes,⁴³ but this study provides valuable insights into the multiple genetic mechanisms influencing lumbar disc disease and its progression.

Key Points

- Twenty-five candidate genes, derived from a study of knee osteoarthritis, were tested for association with lumbar disc disease radiographic progression.

- Progression was measured looking at change in radiographic grade, adjusted for baseline, in disc space narrowing, osteophytes, and Kellgren-Lawrence for L1–L5 in 720 women followed over a 9-year period.
- Genes in inflammatory pathways (COX2, TIMP1, MMP3), vitamin D receptor, and thrombospondin genes were found to be the ones most strongly associated with radiographic progression of lumbar spine disease.



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