Radiographic severity of knee osteoarthritis is conditional on interleukin 1 receptor antagonist gene variations

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ABSTRACT

Background A lack of biomarkers that identify patients at risk for severe osteoarthritis (OA) complicates development of disease-modifying OA drugs.

Objective To determine whether inflammatory genetic markers could stratify patients with knee OA into high and low risk for destructive disease.

Methods Genotype associations with knee OA severity were assessed in two Caucasian populations. Fifteen single nucleotide polymorphisms (SNPs) in six inflammatory genes were evaluated for association with radiographic severity and with synovial fluid mediators in a subset of the patients.

Results Interleukin 1 receptor antagonist (IL1RN) SNPs (rs419598, rs315952 and rs9005) predicted Kellgren–Lawrence scores independently in each population. One IL1RN haplotype was associated with lower odds of radiographic severity (OR=0.15; 95% CI 0.065 to 0.349; p<0.0001), greater joint space width and lower synovial fluid cytokine levels. Carriage of the IL1RN haplotype influenced the age relationship with severity.

Conclusion IL1RN polymorphisms reproducibly contribute to disease severity in knee OA and may be useful biomarkers for patient selection in disease-modifying OA drug trials.

INTRODUCTION

Osteoarthritis (OA) is characterised by progressive loss of joint articular cartilage and subchondral bone remodelling. Although OA is the greatest cause of disability and much of the population is susceptible, some patients remain relatively stable with minimal change in their symptoms over time. Other patients, however, progress to severe structural deterioration that often leads to disability and joint replacement. One challenge in both clinical management of OA and development of disease-modifying drugs is the lack of imaging or biomarker tools that predict which patients with OA are more likely to progress to severe disease. Genetics explain substantial OA variance,[1] but genetic associations with knee OA severity or progression have not been replicated. Inflammatory mediators regulate breakdown of collagen, proteoglycans and bone that constitute the articular joint tissues and appear to be part of the destructive process in OA. We and others have previously shown that an imbalance in interleukin 1 (IL1) and IL1 antagonists contribute to cartilage loss and increased inflammatory mediators in OA.[2]

Variations in several genes for proteins that regulate inflammation, including interleukin-1α (IL1α), IL1β, IL1 receptor antagonist (IL1Ra), IL10, tumour necrosis factor α (TNFα) and oestrogen receptor-α (ESR1), have been associated with differential expression of inflammatory mediators,[3–5] and most of these gene variations have been associated with susceptibility to OA in various joints.[6–10] In this study, we therefore evaluated whether polymorphisms in selected inflammatory genes and genes that regulate inflammation could stratify patients with knee OA into high and low risk for radiographic severity in two independent populations.

PATIENTS AND METHODS

Patient populations

Two independent populations were recruited at New York University Hospital for Joint Diseases (NYUHJD) and from the Prediction of Osteoarthritis Progression (POP) study (Duke University Medical Center). Institutional review board approval of protocols and informed consent of patients for this study were obtained. All Caucasian patients in the NYUHJD and POP populations that met clinical symptomatic criteria (American College of Rheumatology) and radiographic criteria for OA (Kellgren–Lawrence (KL) grade >1) of at least one knee and were age ≥38 years were included. Patients with histories of corticosteroid use, bilateral knee replacements, other forms of arthritis, cancer or other chronic diseases beyond hypertension or hypercholesterolaemia were excluded. POP included subjects with knee OA of KL grade 1–3 in at least one knee and excluded subjects with bilateral knee KL=4 scores, since the primary goal of the POP study was to evaluate the risk factors for progression and to develop a predictive model. Patient demographics are shown in table 1. Patients underwent standardised fixed-flexion postero-anterior knee radiographs with a positioning frame (SynaFlexer; Synarc, San Francisco, California, USA). Radiographs were scored for KL grade (0–4), medial and lateral joint space width (JSW) at the midportion of the joint space via electronic callipers in the NYUHJD cohort, or minimal JSW with digital callipers (TESA ISO 9001) in the POP cohort.

Genotyping

Blood samples (5 ml) were collected (pyrogen-free heparinised tubes) for DNA extraction. Fifteen single nucleotide polymorphisms (SNPs) in six inflammatory

Table 1 Patient demographics

	NYUHJD	POP study*
Number of patients	80	50
Age (mean±SD)	65.6±9.9	61.2±11.9
Gender (% female)	61.3	82
BMI (mean±SD)	26.4 + 3.7	31.1 + 6.3
Trauma history (%)	20	18
Hand involvement† (%)	53	58

*Duke University Medical Center; †by clinical examination.
BMI, body mass index; NYUHJD, New York University Hospital for Joint Diseases; POP, Prediction of Osteoarthritis Progression study.

response genes, including those for IL1α, IL1β, IL1Ra, TNFα, IL10, oestrogen receptor 1, were genotyped. SNPs genotyped (gene, rs number, frequent nucleotide>less frequent nucleotide, minor allele frequency) were IL1A(+4845), rs17561, G>T, 30.5%; IL1B(−511), rs16944, C>T, 33.4%; IL1B(−1464), rs1143623, G>C, 32.8%; IL1B(−3737), rs4848306, C>T, 44.7%; IL1B(+3954), rs1143634, C>T, 24.2%; IL1B(+3877), rs1143633, A>G, 39.7%; IL1RN(+2018), rs419598, T>C, 27.3%; IL1RN, rs315952, C>T, 25.8%; IL1RN, rs9005, G>A, 30.5%; IL10(−1082), rs1800896, A>G, 46.7%; IL10(−819), rs1800871, T>C, 17.3%; IL10(−592), rs1800872, A>C, 20.2%; TNFA(−308), rs1800629, A>G, 21.7%; ESR1_PvuII, rs2234693, T>C, 44.9%; ESR1_Xbal, rs9340799, A>G, 33.8%. Genotyping was accomplished (Interleukin Genetics Clinical Laboratory, Waltham, Massachusetts, USA; CLIA certified) by PCR targeting the sequence surrounding the SNPs studied. Multiplexed single-base extension reactions were performed. Genotypes were analysed (Beckman Coulter, Brea, California, USA; CEQ8800) and final genotypes were scored by laboratory personnel blinded to all patient data.

Synovial fluid analysis

Synovial fluid (SF) samples were a required component of participation in the POP study, so SF samples were available from all subjects in the POP cohort. Fifty POP subjects met the entrance criteria for this study as described earlier; therefore, the SF samples represented no selection bias. Fluid samples were aspirated directly (n=36) or by lavage (n=14), corrected for dilution by the urea method[11] and analysed blinded to the clinical information. Cytokines (pg/ml) were quantified by multiplex bead assays (Bio-Plex; Bio-Rad, Life Science research, Hercules, California, USA). High-sensitivity C-reactive protein concentrations (mg/l) were measured using the UBI Magiwel Enzyme Immunoassay (United Biotech, Mountain View, California, USA; minimum detectable concentration of 0.00035 mg/l, interassay variation ≤7.4%). Cartilage oligomeric matrix protein (COMP) was measured by sandwich ELISA with monoclonal antibodies 17C10 and 16F12 to human COMP (minimum detection 120 ng/ml, intra-assay and inter-assay variation ≤5.8% and 8.7%, respectively). IL1ra was measured using the Quantikine Human IL1Ra Immunoassay from R&D Systems (Minneapolis, Minnesota, USA), with a minimum detectable dose of 6.26 pg/ml.

Statistical methods

Primary analyses evaluated associations between genotypes and radiographic severity, as measured by KL scores. Patients with KL scores of 1, 2 were compared with those with scores of 3, 4. To determine whether results were potentially due to aberrant KL score distributions, a second analysis was performed comparing KL1 with KL2–4 (results were unchanged). Genotype deviation from Hardy–Weinberg equilibrium was tested (Pearson's χ^2

test) in the control sample. Genotype associations with radiographic severity were determined using χ^2 statistics or Fisher's exact test, adjusted for non-genetic risk factors, age, body mass index (BMI) and gender, where appropriate, using multivariate logistic regression analysis. The effect of multiple comparisons was considered in the discovery population (NYUHJD) involving 15 SNPs. Comparisons of JSW between genotypes were made using non-parametric Wilcoxon test and a mixed model analysis of variance for correlated data to adjust for two knees in the same subject.

RESULTS

The NYUHJD population was analysed first. Patients with more severe knee OA (KL 3, 4 vs 1, 2) were older (p=0.013) but did not differ by gender (p=0.96) or BMI (p=0.46). Subsequent analyses were age adjusted.

All SNPs were in Hardy–Weinberg equilibrium and were evaluated for association with radiographic severity. After adjustment for multiple comparisons, one SNP in the IL1Ra gene (IL1RN) was significantly associated with decreased risk for severe OA and two other IL1RN SNPs showed the same protective trend (table 2). IL10 SNPs, also associated with radiographic severity, were not significant after adjustment for multiple comparisons. We then tested the Duke POP cohort for IL1RN gene variations, based on association with severity in the NYUHJD population. In the POP study, age, gender or BMI were not significant influences, but two of three IL1RN SNPs were significantly associated with knee OA severity (table 2). All other SNPs tested in the NYUHJD population were subsequently evaluated in the POP patients, and none was significant (data not shown).

Since all significant SNPs were in one gene, we evaluated haplotype effects on severity. Of nine possible haplotypes from three IL1RN SNPs, four had a frequency >1%, and one (rs419598/rs315952/rs9005=CTA) was associated with reduced risk for severity in both populations (table 2).

We then combined the two populations for further analyses focusing on the IL1RN loci. In the combined dataset, age was associated with severity (p=0.0065) but gender and BMI were not. IL1RN SNPs and haplotypes were associated with decreased risk for severe disease by KL grade and JSW (table 3). The first JSW analysis was knee based and included the smaller JSW for all knees (n=251 knees with complete data; 126 patients from NYUHJD and Duke POP). JSW analyses were adjusted for age, gender and BMI, and the knee-based analysis used a mixed model analysis of variance for intercorrelated data to adjust for two knees in the same subject. Two IL1RN SNPs were significantly associated with greater mean JSW, as was the IL1RN CTA haplotype (mean JSW in patients carrying the CTA haplotype=3.99 mm±1.77, vs reference haplotypes= 3.14 mm+1.93; p=0.0008). In addition to the IL1RN genotype and haplotype effects observed for the signal knees, a similar IL1RN CTA haplotype effect was seen in the contralateral knees when analysed for KL scores of 3–4 (OR=0.065; 95% CI 0.0076 to 0.55; p=0.0024) and JSW (p=0.008).

In a person-based analysis of signal knees only, all signal knees (n=126 with complete data) from NYUHJD and Duke, were classified as to medial (n=95) or lateral-dominant (n=31) disease. The protective effect was apparent for medial knee OA (mean JSW in patients carrying the CTA haplotype=3.37 mm±1.66, vs reference haplotypes =2.29 mm±1.73; p=0.0054). There were no significant IL1RN genotype associations for the lateral compartment (mean JSW in patients carrying the

Table 2 Genotype association with radiographic severity of knee osteoarthritis (OA) in two populations

Gene	rs Number	Genotypes compared	NYUHJD population (n=80*) Kellgren–Lawrence score >2 (odds ratio (CI))	Duke POP study (n=50) Kellgren–Lawrence score >2 (odds ratio (CI))
IL1A(+4845)	rs17561	GG vs GT/TT	1.26 (0.48 to 3.28); p=0.63	
IL1B(−511)	rs16944	CC vs CT/TT	0.89 (0.32 to 2.47); p=0.82	
IL1B(−1464)	rs1143623	GG vs GC/CC	1.96 (0.75 to 5.11); p=0.17	
IL1B(−3737)	rs4848306	CC vs CT/TT	1.25 (0.45 to 3.48); p=0.68	
IL1B(+3954)	rs1143634	CC vs CT/TT	1.36 (0.53 to 3.47); p=0.52	
IL1B(+3877)	rs1143633	GG vs GA/AA	1.12 (0.43 to 2.91); p=0.81	
IL1RN(+2018)	rs419598	TT vs CT/CC	0.49 (0.175 to 1.37); p=0.174	**0.031 (0.004 to 0.27); p=0.0016†**
IL1RN	rs315952	CC/CT vs TT	0.46 (0.15 to 1.20); p=0.113	0.32 (0.090 to 1.00); p=0.071
IL1RN	rs9005	GG vs GA/AA	**0.25 (0.091 to 0.680); p=0.0067‡**	**0.084 (0.02 to 0.343); p=0.0006†**
IL1RN haplotype	rs419598/rs315952/ rs9005	Haplotype C,T,A (1 or 2 copies) vs no copies	**0.29 (0.09 to 0.93); p=0.037†**	**0.031 (0.004 to 0.270); p=0.0016†**
TNFA(−308)	rs1800629	GG vs GA/AA	1.37 (0.040 to 4.70); p=0.62	
IL10(−1082)	rs1800896	CC vs CT/TT	3.00 (0.87 to 10.34); p=0.081	
IL10(−819)	rs1800871	CC vs CT/TT	3.32 (1.08 to 10.28); p=0.037	
IL10(−592)	rs1800872	CC vs CA/AA	3.32 (1.08 to 10.28)§; p=0.037	
ESR1_PvuII	rs2234693	TT vs CT/CC	0.57 (0.22 to 1.49); p=0.25	
ESR1_XbaI	rs9340799	AA vs AG/GG	0.48 (0.18 to 1.25); p=0.13	

Results in bold indicate significant association with decreased risk for severe OA.

*Number of Caucasian patients with knee osteoarthritis who met inclusion criteria and had complete genotype data.

†IL1RN single nucleotide polymorphisms (SNPs) of interest from the NYUHJD population were then tested in the Duke POP population, and common haplotypes in the IL1RN gene were tested in both populations. p Values <0.05 were considered statistically significant. All other SNPs tested in the NYUHJD population were subsequently evaluated in the POP patients, and none were significant (data not shown).

‡For each single SNP, p values <0.027 were considered statistically significant to account for multiple comparisons in the discovery population (NYUHJD) involving 15 SNPs.

§IL10(−819) and IL10(−592) alleles were completely concordant in this population.

NYUHJD, New York University Hospital for Joint Diseases; POP, Prediction of Osteoarthritis Progression study.

Table 3 Interleukin 1 receptor antagonist (IL1RN) genotype association with two radiographic parameters of severity in the combined populations

Genotype or haplotype	Frequency of indicated genotype or haplotype*	Kellgren–Lawrence score >2 (odds ratio (CI))† (N=130)	Joint space width (JSW in mm)‡ (mean±SD) Test genotype	Reference genotype	p Value§
IL1RN rs419598 CC/TC	0.35	0.22 (0.091 to 0.508); p=0.0005	3.83±1.91 (n=89)	3.19±1.88 (n=162)	0.036
IL1RN rs315952 TT	0.53	0.44 (0.21 to 0.92); p=0.0297	3.69±1.80 (n=132)	3.11±2.01 (n=117)	0.069
IL1RN rs9005 AA/GA	0.45	0.15 (0.065 to 0.349); p<0.0001	3.86±1.71 (n=114)	3.04±2.01 (n=135)	0.0063
IL1RN rs419598/ rs315952/rs9005 Carriage of Haplotype C,T,A	0.32	0.14 (0.053 to 0.368); p< 0.0001	3.99±1.77 (n=80)	3.14±1.93 (n=169)	0.0008

*Frequency within the total patients (n=130 with complete genetic data) with knee osteoarthritis (OA) from two separate populations (NYUHJD; Duke POP Study).

†Odds ratio for severe OA, as measured by Kellgren–Lawrence (KL) score, comparing the indicated genotype or haplotype with all other genotypes for that single nucleotide polymorphism. Patients were classified by radiographic severity of knee OA by stratifying the KL scores to compare KL=1 or 2 vs KL=3 or 4. Similar significant associations were found when patients were stratified so that KL=1 was compared with KL=2–4 (data not shown).

‡The smaller JSW in millimetres for each knee was included in the knee-based analysis (n=251 knees with complete data in 126 patients from the NYUHJD and Duke POP populations). Statistical analysis was adjusted for age, gender and body mass index in a mixed model analysis of variance for intercorrelated data to adjust for the subject effect on two knees.

§Wilcoxon test.

NYUHJD, New York University Hospital for Joint Diseases; POP, Prediction of Osteoarthritis Progression study.

CTA haplotype=2.74 mm±2.21, vs reference haplotypes=2.23 mm±1.67; p=0.53).

Since age has strong epidemiological associations with prevalence and severity of knee OA and was associated with severity in the combined studies, we evaluated the interaction between age and IL1RN genotype relative to severity. In regression models, there was a significant interaction between genotype and age (p<0.0001) relative to KL scores, with the effect of genotype being stronger at greater ages. The addition of the IL1RN haplotype to regression models that included age provided a better fit than with age alone (p<0.0001). To examine the effect of genotype at different ages, we divided the population into age tertiles. Carriers of the CTA IL1RN haplotype were at significantly lower risk for severe KL scores in each age group (figure 1A). In regression models, age was associated with KL>2 in patients without the CTA haplotype (p=0.032) but not in those carrying the haplotype. Similarly, age was associated with JSW narrowing only in patients with knee OA who do not carry the CTA haplotype (figure 1B vs figure 1C).

Given that IL1RN alleles have been associated with different expression levels of inflammatory mediators, we evaluated the influence of the IL1RN CTA haplotype on inflammatory mediators in SF from the POP study (n=50). Patients carrying the IL1RN CTA haplotype had significantly lower SF mean levels of IL10 (p=0.034), and showed a trend towards lower levels of IL1β and IL6 (table 4). No differences by IL1RN haplotype were seen for IL1Ra protein, monocyte chemotactic protein-1, macrophage inflammatory protein-1β, C-reactive protein or COMP (table 4). IL1Ra protein levels were positively correlated with
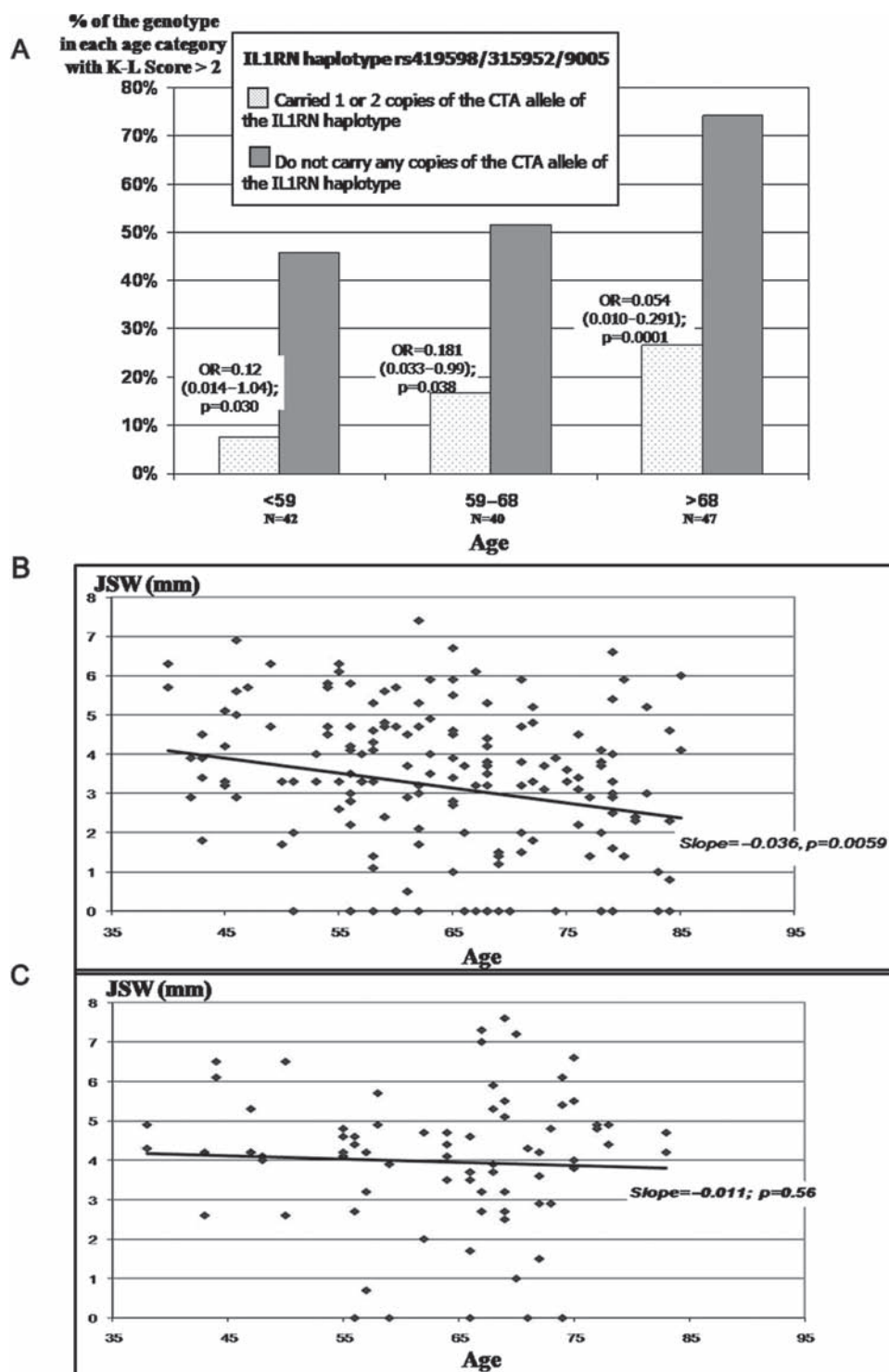
Figure 1 Influence of interleukin 1 receptor antagonist (IL1RN) haplotypes on the age relationship to severity of knee osteoarthritis (OA). (A) The figure stratifies patients with knee OA into thirds by age (<59, n=42; 59–68, n=40; >68, n=47). The numbers in each group are not exactly equal owing to the distribution of patients at the intersection of the groups. Patients within each age group were then stratified by carriage (dot-filled bars) or no carriage (grey-filled bars) of the IL1RN haplotype CTA of the single nucleotide polymorphisms (SNPs) rs419598/rs315952/rs9005. For each age strata and haplotype status, the frequency of severe radiographic knee OA (Kellgren–Lawrence (K–L) score >2) is plotted. (B, C) The joint space width (JSW) of each knee in patients with knee OA who do not (B) or do carry (C) the IL1RN CTA haplotype (rs419598/rs315952/rs9005) is plotted relative to age, and the regression line is shown for JSW relative to age. The JSW is significantly associated with age (p=0.0059) in patients with knee OA who do not carry the IL1RN CTA haplotype, but not associated with age (p=0.56) in patients who carried one or two copies of the CTA haplotype.

IL6 levels both in patients without the CTA haplotype (p=0.002 with exclusion of one outlier; mixed model considering two knees from an individual: p=0.04) and in patients with the CTA haplotype (p=0.029; mixed model: p=0.078). There was a positive correlation between levels of IL10 and IL1ra levels only

in samples from patients without the IL1RN CTA haplotype (p=0.0048; mixed model: p=0.056). No association was seen between levels of IL10 and IL1ra levels in patients who carry the IL1RN CTA haplotype (p=0.37; mixed model: p=0.55) This effect appears to be due in part to the fact that in the lower

Table 4 Synovial fluid inflammatory mediators relative to carriage of interleukin 1 receptor antagonist (IL1RN) haplotypes

Synovial fluid analyte	IL1RN haplotype (rs419598/rs315952/ rs9005=CTA)	N*	Mean†	SD	p Value‡
CRP (ng/ml)	0	28	5195.3	4991.4	0.74
	1 or 2 copies	21	5160.4	4045.8	
IL6 (pg/ml)	0	28	687.1	633.5	0.38
	1 or 2 copies	19	430.2	437.4	
IL10§ (pg/ml)	0	28	109.4	222.7	0.034
	1 or 2 copies	19	13.4	42.7	
IL1β§ (pg/ml)	0	28	3.46	8.38	0.40
	1 or 2 copies	19	0.63	2.04	
IL1Ra¶ (pg/ml)	0	16	483	334.6	0.72
	1 or 2 copies	7	468.5	184.2	
MCP (pg/ml)	0	29	763.7	389	0.49
	1 or 2 copies	21	789.7	303.3	
MIP (pg/ml)	0	29	380.9	233.4	0.91
	1 or 2 copies	21	360.6	169.1	
COMP (ng/ml)	0	29	35020	23280	0.52
	1 or 2 copies	21	30441	20677	

*Number of patients.
†The mean of the mediator values of the two knees was used to represent the mediator value for the patient.
‡Non-parametric Wilcoxon test.
§Values of these parameters that were below the lower limit of detection of the assay were assigned the value of 0.5 times the lower limit to facilitate data analysis. Fifty-two per cent of the IL10 values and 82% of the IL1β values were below the detection threshold limit.
¶IalIL1Ra analysis was performed at a later time than analysis of the other analytes and fewer samples remained available with sufficient volume for analysis.
COMP, cartilage oligomeric matrix protein; IL1, interleukin 1; IL1Ra, interleukin 1 receptor antagonist; MCP, monocyte chemotactic protein-1; MIP, macrophage inflammatory protein-1β.

levels of IL10 (tertiles 1 and 2) IL1Ra is increased in CTA carriers, as shown by the enrichment of CTA haplotype carriers compared with non-carriers in the highest quartile of IL1Ra (54.5% vs 16.7%; p=0.07).

DISCUSSION

Inflammatory mediators are produced by articular joint tissues in OA and have been implicated in disease pathogenesis.[2][12] Of 15 SNPs in six inflammatory genes, variations in the gene for IL1Ra were significantly associated with severe radiographic knee OA in two independent populations. One IL1RN haplotype was associated with decreased risk for radiographic severity, greater JSW, lower levels of SF inflammatory mediators and reduction of the age influence on severity. Although both populations were modest in size, these findings are unlikely to be false positives because (*a*) statistically significant associations were observed for multiple SNPs in the same gene, IL1RN, whereas no associations were found for SNPs in other genes; (*b*) a specific IL1RN haplotype was protective in two independent populations and with two different severity parameters; (*c*) in the combined dataset, associations with severity were highly significant (p<0.0001).

It is of interest that the IL1RN protective effect was only evident in the medial compartment. Although power to show a protective effect for lateral knee OA dominant disease was less, these findings are consistent with previous reports of a stronger genetic effect on medial disease. In a female twins study[1] changes over time in the medial compartment were clearly heritable but changes in the lateral compartment were not, and an MRI-based study[13] also found a stronger genetic influence for medial cartilage loss. The differential compartment effects

observed for genetic influence can presumably be attributed to the combination of genetic susceptibility and weightbearing load (70% concentrated in the medial compartment) which together are necessary and sufficient to cause OA preferentially in the medial compartment.

IL1 is well established as a regulator of cartilage degradation[14] and resorption of bone.[12] We and others have previously shown that imbalances in IL1 and IL1 antagonists, such as the IL1Ra and the type II decoy receptor, contribute to cartilage loss and increased inflammatory mediators in OA.[2] Although blocking IL1 activity by intra-articular injections or by gene transfer of IL1Ra reduced cartilage and bone changes in animal models of OA,[15][16] intra-articular IL1Ra injections in humans have produced mixed results for symptomatic knee OA.[17]

The IL1 gene cluster region has been repeatedly associated with susceptibility to OA in various joints, but the results have been inconsistent.[7–9][18–20] An extended haplotype including SNPs in the IL1A, IL1B and IL1RN genes has been associated with increased susceptibility to OA in various joints,[9][19] but that haplotype is not consistent with the IL1RN haplotypes that contributed to knee OA severity in this study.

Of the three IL1RN SNPs associated with severity in this study, two (rs419598, exon 3 Ala/Ala; rs315952, exon 5, Ser/Ser) are in the coding regions of the IL1RN gene but do not change the amino acid sequence. The third, rs9005, is in the 3'-UTR of exon 4. Blood levels of IL1Ra have been associated with various polymorphic loci within the IL1RN gene, but the findings have been inconsistent. In two recent studies of more than 1000 subjects,[5][21] significantly higher blood levels of IL1Ra protein were associated with allele T at one polymorphism (rs4251961) that is part of the broader haplotype marked by the CTA alleles included in this report. In addition, allele C at rs419598 of the CTA haplotype has been associated with increased peripheral blood mononuclear cell expression of IL1Ra and decreased risk for osteolysis after total hip arthroplasty.[22]

IL1β is among the first genes activated with any tissue challenge, including biomechanical stress, and activates downstream cytokines including IL6 and IL10. The lower SF levels of IL10, IL1β and IL6 in the presence of the IL1RN CTA haplotype are consistent with lower IL1 biological activity, as would be expected in individuals with increased IL1Ra expression and a lower level of activation of local cytokine-producing cells.

Although no simple relationship was seen between the SF levels of IL1Ra and carriage of the IL1RN CTA haplotype in all patients, this relationship was evident in patients who were not in the highest tertile of IL10 levels. IL1Ra expression is directly activated by IL10 in both monocytes and neutrophils.[23][24] The finding of a higher level of ILRa in SF samples from patients carrying the CTA haplotype only in the lower two tertiles of IL10 protein is consistent with a genetic effect that may be evident only under submaximal activation conditions. For example, if one assumes near-maximal activation of IL1Ra when IL10 is strongly elevated, as would be expected in tertile 3 of IL10 levels, a genotype effect on IL1ra expression may be less evident than at submaximal levels of IL10.

This study has potential limitations, including the moderate sample size, yet consistent and significant severity associations with the IL1RN loci were found in two independently ascertained populations. In addition, although the genotype association is with severity, longitudinal data are not available to assess actual disease progression rates. In this study, age was associated with radiographic severity, and although adjusted for in the statistical models, age may have had other confounding effects on the results. It should be emphasised that the IL1RN

haplotype that was associated with protection in late stages of a chronic disease that involves both bone and cartilage, may have a different or no effect on early disease initiation.

A substantial part of the variance in clinical expression of OA is attributed to genetics,[1] and multiple SNPs have been associated with OA susceptibility.[25] Few studies have evaluated the role of genetic factors in severity or progression of knee OA,[26 27] and, other than our report, we are not aware of genetic markers for knee OA severity validated in a second population.

The IL1RN CTA haplotype appears to identify a substantial segment of patients with knee OA who are at low risk for severe destruction, and the data suggest that IL1 biological activity is a determinant of knee OA severity. Biomarkers that identify patients more likely to develop severe disease should expedite successful development of disease-modifying OA drugs and improvements in medical and surgical management of knee OA.

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