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

Mukundan Attur,1 Hwa-Ying Wang,2 Virginia Byers Kraus,3 Jack F Bukowski,2,4 Nazneen Aziz,2 Svetlana Krasnokutsky,1 Jonathan Samuels,1 Jeffrey Greenberg,1 Gary McDaniel,3 Steven B Abramson,1 Kenneth S Kornman2

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 ≥58 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 posterior-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
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(rs48454), rs17561, G>T, 30.5%; IL1B(−511), rs16944, C>T, 33.4%; IL1B(−1464), rs1145623, G>C, 32.8%; IL1B(−3757), rs4848306, C>T, 44.7%; IL1B(3954), rs1148634, C>T, 24.2%; IL1B(+3877), rs1148633, A>G, 39.7%; IL1RN(+2018), rs419598, T>C, 27.3%; IL1RN, rs315952, C>T, 25.8%; IL1RN, rs90005, 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_Polvul, 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; CEG8800) 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 and analysed blindly 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). IL1α 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 χ² test) in the control sample. Genotype associations with radiographic severity were determined using χ² 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/rs90005=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

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs Number</th>
<th>Genotypes compared</th>
<th>NYUHJD population (n=80*)</th>
<th>Duke POP study (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1A (+4845)</td>
<td>rs17561</td>
<td>GG vs GT/TT</td>
<td>1.26 (0.48 to 3.28); p=0.63</td>
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<tr>
<td>IL1B (−511)</td>
<td>rs16944</td>
<td>CC vs CT/TT</td>
<td>0.89 (0.32 to 2.47); p=0.82</td>
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<tr>
<td>IL1B (−1464)</td>
<td>rs1143623</td>
<td>GG vs GC/CC</td>
<td>1.96 (0.75 to 5.11); p=0.17</td>
<td></td>
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<tr>
<td>IL1B (−3737)</td>
<td>rs4848306</td>
<td>CC vs CT/TT</td>
<td>1.25 (0.45 to 3.48); p=0.68</td>
<td></td>
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<tr>
<td>IL1B (−3954)</td>
<td>rs1143634</td>
<td>CC vs CT/TT</td>
<td>1.25 (0.53 to 3.47); p=0.52</td>
<td></td>
</tr>
<tr>
<td>IL1B (+3877)</td>
<td>rs1143633</td>
<td>GG vs GA/AA</td>
<td>1.12 (0.43 to 2.91); p=0.81</td>
<td></td>
</tr>
<tr>
<td>IL1RN (2018)</td>
<td>rs419598</td>
<td>TT vs CT/CC</td>
<td>0.49 (0.175 to 1.37); p=0.174</td>
<td>0.031 (0.004 to 0.27); p=0.0016†</td>
</tr>
<tr>
<td>IL1RN</td>
<td>rs315952</td>
<td>CC/CT vs TT</td>
<td>0.46 (0.15 to 1.20); p=0.113</td>
<td>0.32 (0.090 to 1.00); p=0.071</td>
</tr>
<tr>
<td>IL1RN</td>
<td>rs9005</td>
<td>GG vs GA/AA</td>
<td>0.25 (0.091 to 0.680); p=0.0067†</td>
<td>0.084 (0.02 to 0.343); p=0.00061‡</td>
</tr>
<tr>
<td>IL1RN haplotype</td>
<td>rs419598/rs315952/rs9005</td>
<td>Haplotype C,T,A (1 or 2 copies) vs no copies</td>
<td>0.29 (0.09 to 0.93); p=0.037†</td>
<td>0.031 (0.004 to 0.270); p=0.0016†</td>
</tr>
<tr>
<td>TNFA (−308)</td>
<td>rs1800629</td>
<td>GG vs GA/AA</td>
<td>1.37 (0.040 to 4.70); p=0.62</td>
<td></td>
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<tr>
<td>IL10 (−1082)</td>
<td>rs1800896</td>
<td>CC vs CT/TT</td>
<td>3.00 (0.87 to 10.34); p=0.081</td>
<td></td>
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<tr>
<td>IL10 (−819)</td>
<td>rs1800871</td>
<td>CC vs CT/TT</td>
<td>3.32 (1.08 to 10.28); p=0.037</td>
<td></td>
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<tr>
<td>IL10 (−592)</td>
<td>rs1800872</td>
<td>CC vs CA/AA</td>
<td>3.32 (1.08 to 10.28); p=0.037</td>
<td></td>
</tr>
<tr>
<td>ESR1_Pvul</td>
<td>rs2234693</td>
<td>TT vs CT/CC</td>
<td>0.57 (0.22 to 1.49); p=0.25</td>
<td></td>
</tr>
<tr>
<td>ESR1_Xba</td>
<td>rs9340799</td>
<td>AA vs AG/GG</td>
<td>0.48 (0.18 to 1.25); p=0.13</td>
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</table>

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...
These findings are consistent with previous reports of a strong effect in the medial compartment. Although power to show a true association was limited by the sample size, yet consistent and significant severity associations were found for SNPs in the IL1RN gene. In addition, although the genotype association is with severity, longitudinal data are not available to confirm the association is with severity, longitudinal data are not available to confirm the association is with severity, longitudinal data are not available to confirm the association is with severity, longitudinal data are not available to confirm the association is with severity, longitudinal data are not available to confirm the association is with severity, longitudinal data are not available to confirm the association is with severity, longitudinal data are not available to confirm the association is with severity, longitudinal data are not available to confirm the association is with severity, longitudinal data are not available to confirm the association is with severity, longitudinal data are not available to confirm the association is with severity, longitudinal data are not available to confirm the association is with severity, longitudinal data are not available to 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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, and multiple SNPs have been associated with OA susceptibility. Few studies have evaluated the role of genetic factors in severity or progression of knee OA, 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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Competing interests When the research was conducted JFB, NA and KSK were full-time employees and H-YW was a part-time consultant for Interleukin Genetics.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the NYU institutional review board.

Contributors The study concept, design was developed by SBA, KK, JB and MA. Data and statistical analysis was performed by NA, KK, VK and H-YH. Patient recruitment and clinical data collection were performed by SK, JS, JGB, GM and VK. The manuscript was drafted and critically checked by KK, NA, SA, VK and MA.

Provenance and peer review Not commissioned; externally peer reviewed.

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