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IL-1 receptor antagonist gene as a predictive biomarker of progression of knee osteoarthritis in a population cohort

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Abstract

Objective—Within the interleukin-1 (IL-1) cytokine family, IL-1 receptor antagonist (IL1RN) gene variants have been associated with radiological severity of knee osteoarthritis (OA) in cross-sectional studies. The present study tested the relation between IL1RN gene variants and progression of knee OA assessed radiographically by change in Kellgren-Lawrence (KL) score over time.

Design—1153 Caucasian adults (age range 44-89) from the Johnson County Osteoarthritis Project were evaluated for unequivocal radiographic evidence of knee OA at baseline, defined as KL score ≥ 2, and were re-examined after 4-11 years for radiographic changes typical of OA progression. IL1RN gene variants were tested for association with OA progression and for

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Conflict of interest X. Wu, V. Kondragunta, and K.S. Kornman are current or past employees and stockholders of Interleukin Genetics, Inc. H-Y. Wang is a statistical consultant for Interleukin Genetics, and G.W. Duff is a scientific advisor and stockholder of Interleukin Genetics. J.M. Jordan is a consultant and has stock options in Algynomics, Inc, a company interested in examining genetics of pain in multiple genetic conditions.

Contributions XW: participated in study design, managed overall project execution, oversaw quality assurance of genetics database, participated in data interpretation and manuscript drafting
VK: participated in study design, data analysis and interpretation, and manuscript drafting
KSK: participated in study design, participated in data interpretation, planned and co-drafted first version of manuscript, and participated in major manuscript revisions
HW: participated in data analysis and interpretation
GWD: participated in study design, participated in data interpretation, planned and co-drafted first version of manuscript, and participated in major manuscript revisions
JBR: participated in study design, radiographic analysis and interpretation
JMJ: participated in study design, participated in data interpretation, participated in manuscript drafting and major manuscript revisions
All authors read and approved the final manuscript.

potential interaction with body mass index (BMI). Other IL-1 gene variations were tested for association with OA progression as a secondary objective.

Results—Of 154 subjects with OA at baseline, 88 showed progression at follow-up. Seven IL1RN single nucleotide polymorphisms (SNPs) and one IL-1 receptor SNP were associated with progression. Four IL1RN haplotypes, each occurring in >5% of this population, showed different relationships with progression, including one (rs315931/rs4251961/rs2637988/rs3181052/ rs1794066/rs419598/rs380092/ rs579543/rs315952/rs9005/rs315943/rs1374281; ACAGATACTGCC) associated with increased progression (OR 1.91 (95%CI 1.16-3.15), p = 0.012). Haplotypes associated with progression by KL score were also associated with categorical change in joint space narrowing. BMI was associated with OA progression in subjects carrying a specific IL1RN haplotype, but not in subjects without that haplotype.

Conclusion—A significantly greater likelihood of radiological progression of knee OA was associated with a commonly occurring IL1RN haplotype that could be tagged by three IL1RN SNPs (rs419598, rs9005, rs315943). Interactions were also observed between IL1RN gene variants and BMI relative to OA progression. This suggests that IL1RN gene markers may be useful in stratifying patients for medical management and drug development.

Keywords

osteoarthritis; progression; Interleukin-1; genetics; population stratification; predictive biomarker

Introduction

Arthritis is one of the leading causes of disability[1] and osteoarthritis (OA), the most common type, affects an estimated 27 million people in the United States[2]. The knee is among the most usual joints affected and was responsible for 686,000 total knee replacements (TKR) in 2009, a cumulative growth of 84% from 1997, making TKR the 14th most common hospital procedure in 2009[3].

There is inter-individual variation in the rate of progression of knee OA, but factors determining progression are poorly understood. Inability to predict which patients with knee OA are likely to exhibit measurable disease progression during a monitoring period has added to the difficulty of developing disease-modifying interventions.

Although twin and family studies point to a significant genetic component [4-7], few gene variants implicated in OA susceptibility or pathogenesis have been replicated independently or proven robust in meta-analyses. The majority of OA genetic studies have focused on disease susceptibility, and phenotype diversity has complicated attempts to synthesize published findings [8]. Few studies have evaluated the genetics of OA progression [5,9-13]. Availability of a longitudinally monitored population cohort provides a reduction in selection biases that may enrich case-control studies for more complex phenotypes and the opportunity to explore risk factors that differentiate progressive disease at a stage when intervention may be more successful.

The Interleukin-1 (IL-1) family of cytokines has been implicated in the pathogenesis of OA (notably agonist IL-1β and the receptor antagonist IL-1ra), and several IL-1 gene variants have been associated with various OA phenotypes, but meta-analyses have not supported a role for the IL-1 gene family in susceptibility to knee OA [14,15]. Our present study was not concerned with susceptibility to OA, but aimed to detect any relation of IL-1 receptor antagonist gene (IL1RN) variants with the progression of established OA of the knee. Specific haplotypes of IL1RN have previously been associated with radiographic severity of symptomatic knee OA in clinic populations [16]. The current study used a well-defined

population cohort sample from the Johnston County Osteoarthritis Project to test IL1RN polymorphisms in relation to radiographic progression of knee OA over 4 to 11 years. In addition, other variations in the IL-1 gene cluster were evaluated for their potential link with OA progression.

Materials and Methods

Study subjects and Radiographic Assessments

This study examined radiographic progression of knee osteoarthritis using data from the Johnston County (JoCo) Osteoarthritis Project, a population based study of OA in Johnston County, NC, USA. Institutional Review Boards of the Centers for Disease Control and Prevention and the University of North Carolina School of Medicine approved the protocol, and all subjects provided written consent for analysis of genetic and non-genetic biomarkers.

Details of the population cohort study have been reported previously [17]. The JoCo study was designed to be representative of the civilian, non-institutionalized English-speaking black and white population age >=45 years. Baseline examination of subjects was conducted between 1990 and 1997, and subjects were re-examined 4 to 11 years later. Anterior-posterior standing knee radiographs were obtained with footmat positioning at both time points and read by a single musculoskeletal radiologist for Kellgren-Lawrence score (KL, 0-4)[18]. KL scores are the most commonly used measures in population-based studies of radiographic knee OA [19]. A KL score of >=2 indicates at least mild radiographic OA[20]. X-rays were also evaluated for presence and severity of joint space narrowing, scored 0-3 (0=none, 1= mild or > 0 and up to 33% JSN, 2= moderate or > 33% and up to 66% JSN, and 3= severe or from > 66 to 100% JSN), using the Burnett atlas[21]. Demographic and clinical characteristics, such as sex, race, education, history of knee injury, and presence of knee symptoms, were assessed in an interviewer-administered questionnaire. A total of 3,187 and 1,733 eligible subjects completed both home interviews and clinical examinations at baseline and first follow up, respectively.

All Caucasian subjects in the JoCo cohort were selected for inclusion if they completed clinical examinations and home interviews at both baseline and follow up, and had blood samples available for DNA extraction. These included a total of 1,172 subjects, of which 19 were excluded prior to analysis because of questionable gender or ethnicity designations.

All subjects who had radiographic knee OA at baseline (KL>=2) were divided into either progressors (cases) or non-progressors (controls). Progressors had an increase in KL score at follow-up, whereas non-progressors did not.

Genetic variations

We analyzed 21 SNPs: 2 SNPs in IL-1α (IL1A) gene, 6 SNPs in IL-1β (IL1B) gene, 1 SNP in the IL1R1 gene, and 12 SNPs in the IL-1 receptor antagonist (IL1RN) gene on chromosome 2q13 (Table 1). The 12 assayed IL1RN SNPs included seven IL1RN gene tag SNPs that capture (r^2 >0.80) 94% of the Caucasian European HapMap SNPs with minor allele frequency >5% (HapMap release 24, CEU population) [22]and an additional 5 IL1RN SNPs that have been reported in the OA literature.

Genotyping

All genotyping was performed in a CLIA certified molecular genetics laboratory (Interleukin Genetics, Waltham MA). DNA was extracted from blood samples using standard protocols. Before polymerase chain reactions (PCR), DNA was diluted to adjust concentrations to within a range compatible with multiplex PCR conditions. SNP

genotyping was by the single-base extension (SBE) method using the SNPstream instrument and chemistry (Beckman Coulter; Brea, CA) with SNPs multiplexed as needed to avoid interference. The SBE reaction was performed per manufacturer's protocol, and "tagged" products were hybridized to a 48-plex microarray plate. Plates were read by the SNPstream instrument and SNPstream software determined initial allele calls, which were confirmed by a technologist. We validated the assay accuracy and reproducibility of genotyping assays for all SNPs prior to genotyping all study samples. Each validation plate (384 wells) included negative controls (water, n=10), positive controls of known genotypes (Coriell, n=16), and a subset of study samples (n=110). Positive controls and study samples were genotyped in duplicate or triplicate.

Statistical Methods

Genotyping performance was assessed as total genotyping success rate, genotype concordance rate between duplicates, and Hardy-Weinberg Equilibrium (HWE). SNPs with minor allele frequencies <0.01 were excluded. Extent of linkage disequilibrium (LD) was assessed as r^2 using Haploview [23] version 3.3. Logistic regression analysis[24] was applied to examine association between SNP variants and OA progression (increase in KL score from baseline to follow up). Nonparametric Wilcoxon-Mann-Whitney test[25,26] was used to compare OA progression rates between subjects with high BMI (>median) and subjects with low BMI (<=median).

A backward elimination method[27] was used to identify the most efficient set of tag SNPs for IL1RN haplotypes associated with OA progression. The 12 IL1RN SNPs were used to produce the first haplotype model. Then each of the 12 SNPs was removed to create twelve 11 SNP models. Of these, the one with the lowest associated p value was deemed the best model. Each SNP was removed from this best 11-SNP model to create eleven 10 SNP models. Again, the one with the lowest p value was considered the best 10-SNP model. This process was repeated to identify the best 9-SNP, 8-SNP, 7-SNP, 6-SNP, 5-SNP, 4-SNP, 3-SNP, 2-SNP and 1-SNP models. For each model ,we used Haplo.stat [28] to estimate haplotype frequencies for cases and controls and to estimate an odds ratio for each individual haplotype to determine whether haplotypes differed significantly between cases and controls. Overall, 77 tests were evaluated in the backward elimination models to determine which had the lowest p-value. Bonferroni correction was used to adjust for multiple testing, and a specific model was considered significant if it had a p value <0.000649. Alternatively, false discovery rate[29] was used to correct multiple comparisons.

We used a conditional haplotype analysis[30] (WHAP) to determine which of the 12 IL1RN SNPs were responsible for haplotype association with progression observed in this sample. Statistical software, including PLINK[31], JMP genomics (SAS Institute Inc., Cary, NC, 1989-2007.), and SAS (SAS Institute Inc., Cary, NC, 1989-2007), were used in the analysis.

Results

Multiplexed genotyping assays for all of the IL-1 SNPs (Table 1), as described in the Methods section, were 100% accurate for samples of known genotypes, and averaged 99.5% concordance (range: 96.5 to 100.0%) between study sample replicates. Five of the 1153 samples had genotyping failure rates that exceeded quality assurance standards and were excluded from the analysis, leaving 1148 samples with genotyping data for IL-1 SNPs. No major population stratification was detected in this cohort as assessed by GWAS data (data not shown). Pairwise linkage disequilibrium (LD) estimates (r^2) were calculated for the IL1RN SNPs and are shown in Supplemental Figure 1.

Progression

1148 subjects with baseline and follow-up radiographs and genetic data were classified as to presence (KL score of > 2 for at least one knee) or absence of knee OA at baseline. Characteristics of study subjects are shown in Table 2. Additional description of OA progressors can be found in Supplemental Table 1.

Of the 21 IL-1 gene variations analyzed as single SNPs, 7 of the 12 IL1RN SNPs assayed and one in the IL-1 receptor type 1 gene (IL1R1) were significantly associated with radiographic progression of knee OA measured by change in KL score (Table 3). No IL1A or IL1B SNPs were associated with radiographic progression.

Haplotypes

The IL1RN 3-SNP (rs419598/rs315952/rs9005) haplotypes previously associated with knee OA severity [16] were associated with radiographic progression of knee OA in this population cohort (p= 0.0004; Table 4). Haplotype frequencies observed in this Johnston County cohort (n=1,148) were similar to those reported for Utah residents with ancestry from northern and western Europe in the HapMap database (HapMap release 28, CEU population) [22]. The CTA haplotype, previously reported as protective in a cross sectional study of severity, was not associated with disease progression in the present population cohort study (OR=1.16; p=*0.613*) [16], whereas the TTG haplotype was more frequent in progressors and the TCG and TTA haplotypes were more frequent in non-progressors (Table 4).

Since multiple SNPs in the IL1RN gene were associated with progression and the current study provided broad coverage of the genetic variation in the gene, we constructed de novo haplotypes using combinations of 12 IL1RN SNPs.

Of the nine 12-SNP IL1RN haplotypes observed in this population at an estimated frequency of >1%, four were at > 5% (Table 5). Subjects with genetic markers contained in haplotype pattern 1 had an increased likelihood of progression and subjects with pattern 3 appeared to be protected from progression during the follow-up period. Using the Relative Predispostional Effect analysis[32], pattern 1 was shown to confer the largest effect on disease progression while pattern 3 had no effect (Supplemental Table 2). These analyses indicate that one common haplotype (pattern #1) was associated with radiographic progression of the knee.

To determine which combinations of SNPs most efficiently captured the disease related information in the 12-SNP IL1RN haplotypes, we used a backward elimination method, as described in the Methods section. Three SNPs (rs419598/rs9005/rs315943) produced the only significant model after correction for multiple testing as shown in Table 6. Models composed of 4 or 5 SNPs approached but did not reach significance after correction, and no other haplotype models approached significance after correction (Full model in Supplemental Table 3).

In the best 3-SNP model, one specific haplotype (TGC; frequency= 0.41) was significantly associated with increased risk of radiographic progression, two haplotypes (CAT, frequency= 0.26; TGT, frequency=0.28) had no influence on risk for progression, and one haplotype (TAT, frequency=0.04) was associated with decreased risk of radiographic progression (Supplemental Table 4). We then estimated (HaploStat) frequencies of 3-SNP (rs419598/rs9005/rs315943) IL1RN haplotypes in radiographic progressors and non-progressors, as measured by change in KL score and by categorical joint space narrowing (JSN) scores (Table 7; KL change p=0.0003; JSN change p=*0.044*). The haplotype (TGC), which was associated with KL progression in the backward elimination model, was more frequent in KL progressors versus non-progressors (p=*0.006*) and in JSN progressors versus

non-progressors (p=*0.015*). The TGC association with progression remained significant after adjustment for age, gender and BMI (KL progression: odds ratio 2.05, p=0.0059; JSN progression: odds ratio=2.35, p=0.0172). Two other haplotypes were more frequent in KL non-progressors and showed similar, but non-significant, directional associations with JSN progression.

We examined the test performance parameters of the IL1RN gene markers in predicting OA progression (Supplemental Table 5). Depending on the genetic model (dominant or recessive) used, sensitivity of the test ranged from 20.5% to 75%, specificity from 49.2% to 87.7%, positive predictive value from 66.7% to 69.2%, and negative predictive value from 44.9% to 59.3%.

We also evaluated whether there was interaction between the TGC haplotype and BMI, which has been strongly associated with long-term progression of knee OA[33]. The rate of progression was not significantly higher in subjects with a BMI greater than the median (28.5 kg/m^2) compared to those below the median (Supplemental Figure 2). For subjects who did not carry the TGC haplotype, BMI did not influence the progression rate (Supplemental Figure 2; p=0.84) whereas elevated BMI increased progression rate in TGC positive individuals (Supplemental Figure 2; p=0.04).

In addition to defining a subset of SNPs that efficiently tag the broad haplotypes associated with progression, we sought to determine which SNPs may explain IL1RN genetic risk for progression. A conditional haplotype analysis[30] was used to determine which SNPs among the 12 were essential for the observed haplotype association with risk (Table 8). Significant haplotype associations with progression were eliminated by conditioning the model on any one of the five SNPs: rs4251961; rs380092, rs315952, rs315943; and rs1374281, indicating each was essential to the observed significant haplotype associations with progression (Table 8).

Although no individual IL1A or IL1B SNP was significantly associated with radiographic progression in this cohort, some IL1B SNPs (rs16944, rs1143623, rs4848306) have been reported to function in haplotype context [34] to influence clinical levels of IL-1β and C-reactive protein[35]. We evaluated haplotypes for the 8 SNPs covering the IL1A-IL1B region and also for the 3 IL1B promoter SNPs (rs16944, rs1143623, rs4848306). Of the 12 IL1A-IL1B haplotypes observed in >0.01 frequency, only one (TTGTGACG; rs17561/ rs10496444/rs1143643/rs1143634/rs1143633/rs16944/rs1143623/rs4848306) of low frequency (0.024) was significantly associated with radiographic progression after adjustment for age, gender, BMI (Odds Ratio= 0.09; p= 0.0316). No IL1B promoter haplotypes were associated with progression.

Discussion

Osteoarthritis is a complex chronic disease of aging with initiation and progression apparently influenced by multiple factors, including genetics, body weight, trauma, dysplastic tissues and biomechanics[33,36,37]. In spite of practical challenges associated with disease heterogeneity, the ability to predict which OA patients are more likely to exhibit measurable knee OA progression during a defined time period should advance the development of disease modifying drugs.

The primary objective of this study was to test whether IL1RN gene variations previously associated cross-sectionally with knee OA severity [15,16,38] or susceptibility [39,40] might be associated with longitudinally monitored radiographic progression of knee OA in a population cohort. We found a significant association between progression and several SNPs and their haplotypes in the gene for IL-1Ra, indicating that variants of this gene may be

important predictive markers of severity or progression. Four 12-SNP IL1RN haplotypes accounted for 88% of those observed at frequencies > 0.01 in the cohort and one commonly occurring 12-SNP haplotype (Table 5 Pattern 1) was associated with radiographic progression (OR= 1.91; p= 0.012).

The 12-SNP IL1RN haplotypes associated with differential risk for progression were efficiently tagged using 3 specific SNPs (rs419598/rs9005/rs315943). However, multiple combinations of 3 or more SNPs will identify the same set of extended haplotypes, including the 3 SNPs (rs419598/rs315952/rs9005) previously reported to be associated with knee OA severity [16].

To investigate which of the IL1RN SNPs examined in this study contributed to the association with progression observed with the 12-SNP haplotypes, we used a conditional analysis that controlled for each specific SNP. This was accomplished by comparing association with progression for 12-SNP haplotypes with the two different alleles at each conditioning SNP. Loss of haplotype association with progression when conditioning on a specific SNP indicated that the SNP contributed to the association signal, compatible with a mechanistic role in the association for that specific SNP or another in LD with it. With the conditional analysis, only rs4251961, rs380092, rs315952, rs315943, and rs1374281 contributed to the observed association with progression. SNPs that were identified by the backward elimination method were not identical to those identified by conditional analysis to be important for, or potentially causal to, disease progression. This observation is not surprising since the best combination of SNPs that tag haplotypes associated with a trait may not include all SNPs important or causal to the trait. Presumably important/causal SNPs for OA progression identified in this study were highly linked, thus would provide redundant information in tagging haplotypes associated with disease progression and be eliminated in the analysis of the best tagging SNPs.

Using two different analytical methods, SNPs in both LD blocks (supplemental Figure 1) were shown to be associated with radiographic progression of knee OA. This suggests that IL1RN gene or another gene tagged by these IL1RN SNP markers may contribute to the pathogenesis of OA or its progression. SNPs in LD block 1 (Supplemental Figure 1) that best tagged the predominant haplotypes (rs419598) or were identified in the conditional analysis (rs4251961) have been reported to have genotype-specific biological activity. In a study of osteolysis after total hip arthroplasty, allele C at rs419598, (a tag of haplotype #2;Table 5), was associated with reduced risk for osteolysis (OR=0.66; p=0.012)[41]; and stimulated peripheral blood mononuclear cells from subjects with rs419598 genotype CC had increased IL-1Ra mRNA expression compared with genotype TT. In European and American Caucasian populations, allele C of the promoter polymorphism rs4251961, that tags IL1RN haplotype #1 (Table 5), has been associated with lower blood levels of IL-1Ra [42,43], but the allele-specific effects on IL-1Ra were reversed in sub-Saharan Africans [44]. The other LD block includes two of the three haplotype tags (rs9005 and rs315943) and two of the five SNPs identified in the conditional analysis (rs315943 and rs315952). Most of these SNPs are in the 3′ untranslated region of the IL1RN gene which has been reported to have a post-transcriptional influence on IL-1Ra levels [45].

In a previous report [16] IL1RN haplotypes defined by 3-SNPs (rs419598/rs315952/rs9005) were associated with radiographic severity of knee OA, with the CTA haplotype having lower frequency in patients with severe knee OA (KL=3,4) than those with less severe radiographic OA (KL=1,2). In the present study, although the 3-SNP haplotypes defined by rs419598, rs315952 and rs9005 were differentially associated with progression (Table 4), the specific CTA haplotype showed no relationship to knee OA progression (OR= 1.16; p= 0.613). The CTA haplotype findings in the two studies may be consistent given the prior

report included symptomatic patients from hospital rheumatology clinics with radiographic knee OA, as opposed to the current population cohort in which knee OA was identified radiographically without regard to symptoms. The haplotype frequencies observed in the Johnston County cohort (Table 4) were similar to those reported for European heritage Caucasians in the HapMap database (HapMap release 28, CEU population) [22]. In the population cohort, the differences in haplotype frequencies between the non-progressors and progressors may be reasonably interpreted to represent biological differences attributable to the haplotypes that influence the disease. In the cross-sectional comparison of severity, the differences in haplotype frequencies may be a function of both biological differences directly related to severity as well as depletion/enrichment effects of selection bias in a symptomatic clinic population. If the CTA haplotype has a neutral biological effect on progression, it may well be depleted in a population that presents with symptomatic disease.

Of interest was the observed interaction between the IL1RN gene variations and BMI, such that the BMI association with progression was only evident in subjects who carried the TGC haplotype (rs419598/rs9005/rs315943). This observation must be explored in larger populations, but if validated represents a refinement of our understanding of mechanisms involved in body weight influences on joint tissues.

In addition to haplotypes and single SNPs in the gene for IL-1Ra, a SNP in the gene for the IL-1 type 1 receptor (IL1R1) was the only other IL-1 family SNP associated with progression. IL-1α and IL-1β agonists both bind IL-1R1 to initiate receptor signaling and IL-1 biologic activities, and IL-1Ra blocks activity by competitively binding the IL-1R1 sites without initiating signal transduction. Variations in the regulatory region of IL1R1 have been associated with plasma IL-1R1 levels [46], and the IL1R1 SNP associated with progression in this cohort was previously associated with severe hand OA [47].

The current study has the strengths of a well-defined phenotype of radiographic progression of knee OA in a longitudinally monitored population cohort of Caucasians. The challenges of defining OA and its heterogeneity, and potential implications of not doing so, have been discussed recently and are likely especially important for genetic studies [8,48]. To assess the potential distinctions between the genetics of progression and susceptibility in the current cohort, we compared 396 control subjects for which both knees had a KL=0 to 359 cases with a KL>2 for at least one knee or knee replacement. Subjects with a maximum KL=1 at one or both knees were excluded from analysis. It is of interest that when subjects who were classified as having radiographic knee OA were compared with those without knee OA, none of the 12 IL1RN SNPs were significantly associated with OA susceptibility in this population (data not shown).

Few studies have evaluated the genetics of OA progression [5,9-13]. Advantages of a longitudinally monitored population cohort include a reduction in selection biases that may enrich case-control studies for more complex phenotypes, and the opportunity to explore risk factors that differentiate progressive disease at a stage when intervention may be more successful. By limiting the population to Caucasians, we reduced the variation in haplotype frequencies among ethnicities that may complicate data interpretations due to inadvertent enrichment of cases or controls. IL1RN haplotype frequencies observed in the current study were similar to those reported for Caucasians in public databases (HapMap release 28, CEU population) [22]. The use of categorical radiographic parameters to assess disease progression has some limitations, but we used a standardized imaging protocol for all subjects at all time points and radiographs were scored by a calibrated examiner with no knowledge of the genetics.

In conclusion, in this single study of limited sample size, we found that commonly occurring IL1RN haplotypes were associated with radiographic progression of knee OA in a population cohort of Caucasians. The predominant haplotypes that differentiated risk could be efficiently tagged by three SNPs (rs419598, rs9005, rs315943); and a conditional haplotype analysis indicates that of the 12 IL1RN SNPs assayed, five SNPs (rs4251961, rs380092, rs315952, rs315943, rs11374281) in two distinct LD blocks are required for the observed haplotype association with progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Allele and genotype frequencies of SNPs in the IL-1 genes assayed in this study

						Frequency in control				Frequency in case			
Chromosome	**Gene**	**SNP**	**Chromosomal position**	**Alleles**	**Minor allele**	**Minor allele**	**Major homozygote**	**Heterozygote**	**Minor homozygote**	**Minor allele**	**Major homozygote**	**Heterozygote**	**Minor homozygote**
2	IL1R1	rs2287047	102774054	T/C	T	0.19	0.66	0.29	0.05	0.29	0.49	0.44	0.07
2	IL1A	rs17561	113537223	T/G	T	0.35	0.45	0.42	0.14	0.27	0.53	0.40	0.07
2	IL1A	rs10496444	113553668	T/C	C	0.29	0.51	0.40	0.09	0.30	0.48	0.44	0.08
2	IL1B	rs1143643	113588302	A/G	A	0.42	0.35	0.46	0.18	0.41	0.38	0.43	0.19
2	IL1B	rs1143634	113590390	T/C	T	0.23	0.58	0.37	0.05	0.20	0.67	0.26	0.07
2	IL1B	rs1143633	113590467	A/G	A	0.42	0.35	0.46	0.18	0.41	0.38	0.43	0.19
2	IL1B	rs16944	113594867	A/G	A	0.32	0.42	0.54	0.05	0.32	0.43	0.49	0.08
2	IL1B	rs1143623	113595829	C/G	C	0.28	0.48	0.48	0.05	0.26	0.53	0.42	0.05
2	IL1B	rs4848306	113598107	A/G	A	0.50	0.26	0.48	0.26	0.45	0.34	0.41	0.25
2	IL1RN	rs315931	113869843	C/A	C	0.30	0.49	0.42	0.09	0.30	0.47	0.48	0.06
2	IL1RN	rs4251961	113874467	T/C	C	0.34	0.49	0.34	0.17	0.45	0.26	0.57	0.17
2	IL1RN	rs2637988	113876779	A/G	G	0.41	0.42	0.35	0.23	0.38	0.35	0.53	0.11
2	IL1RN	rs3181052	113886049	A/G	A	0.19	0.69	0.24	0.06	0.14	0.74	0.23	0.02
2	IL1RN	rs1794066	113886350	A/G	G	0.44	0.37	0.38	0.25	0.40	0.30	0.60	0.10
2	IL1RN	rs419598	113887207	T/C	C	0.25	0.57	0.37	0.06	0.27	0.48	0.51	0.01
2	IL1RN	rs380092	113888900	T/A	T	0.41	0.34	0.51	0.15	0.27	0.53	0.40	0.07
2	IL1RN	rs579543	113889631	T/C	T	0.28	0.51	0.42	0.08	0.26	0.49	0.50	0.01
2	IL1RN	rs315952	113890304	T/C	C	0.37	0.40	0.46	0.14	0.26	0.56	0.38	0.07
2	IL1RN	rs9005	113891412	A/G	A	0.32	0.48	0.42	0.11	0.27	0.48	0.50	0.02
2	IL1RN	rs315943	113894338	T/C	C	0.32	0.49	0.38	0.12	0.48	0.25	0.55	0.20
2	IL1RN	rs1374281	113898789	C/G	C	0.31	0.51	0.37	0.12	0.48	0.26	0.52	0.22

Control: OA non-progressors (n=66); case: OA progressors (n=88).

Chromosomal positions are derived from Genome Reference Consortium Human Build 37 patch release 5 (GRCh37.p5)[49].

Table2

Characteristics of study subjects

	Total cohort (n=1,148)	Baseline KL≥2 (n=154)	Radiographic Progression (n=88)	No Radiographic Progression (n=66)
	Mean/Freq (SD)	Mean/Freq (SD)	Mean/Freq (SD)	Mean/Freq (SD)
Age	60.3(9.5)	64.5(10.6)	64.6(10.6)	64.5(10.6)
BMI	28.3(5.2)	30.0(6.3)	30.8(6.5)	28.8(5.9)
Gender (Male)	38%	35%	33%	38%

Table 3

SNPs associated with progression of radiographic knee OA: change of KL score in subjects with baseline KL≥2

Gene	SNP[a]	Associated Allele /Genotype	Genetic Model	Odds Ratio	95% CI	p value[b]	q value (False Discovery Rate)
IL1R1	rs2287047	T	Dominant	2.34	1.16-4.74	0.018	0.075
IL1RN	rs4251961	C	Dominant	3.06	1.49-6.26	0.002	0.016
IL1RN	rs2637988	A	Dominant	2.97	1.17-7.49	0.021	0.075
IL1RN	rs1794066	A	Dominant	3.58	1.40-9.16	0.008	0.041
IL1RN	RS380092	T	Dominant	0.44	0.22-0.87	0.019	0.389
IL1RN	RS315952	C	Dominant	0.49	0.25-0.97	0.039	0.415
IL1RN	rs315943	C	Dominant	3.06	1.50-6.23	0.002	0.016
IL1RN	rs1374281	C	Dominant	3.11	1.53-6.31	0.002	0.016

[a] Single nucleotide polymorphism (SNP) Reference sequence number in dbSNP[50]

[b] Logistic regression modeling for individual SNPs are shown with adjustments for age, BMI, and gender LD (r2) between adjacent SNPs in the IL1RN gene: 0.38 for rs4251961 and rs2637988; 0.93 for rs2637988 and rs1794066; 0 for rs1794066 and rs380092; 0.78 for rs380092 and rs315952; 0.27 for rs315952 and rs315943; 0.86 for rs315943 and rs1374281

Table 4

IL1RN haplotypes previously associated with knee OA severity were associated with OA progression

IL1RN SNPs in Haplotype	Haplotype	Freq in JoCo cohort[1]	Freq in HapMap[2]	Freq in progressors	Freq in non-progressors	Unadjusted p-value	Adjusted OR	Adjusted p-value
rs419598/ rs315952/ rs9005	TTG	0.42	0.42	0.47	0.32	*0.0075*	1.96	*0.009*
	TCG	0.27	0.25	0.26	0.37	*0.037*	0.57	*0.037*
	CTA	0.26	0.27	0.27	0.24	*0.535*	1.16	*0.613*
	TTA	0.04	0.04	0.01	0.08	*0.012*	0.08	*0.017*
	Omnibus					0.0004		

[1] Caucasians in the Johnston County Osteoarthritis Project[17]; n=1,148

[2] HapMap (HapMap release 28, CEU population)[22]

Table 5

IL1RN Haplotypes and radiographic progression of knee OA: change of KL Score

Pattern	Haplotype*	Frequency			OR	95%CI	p
		All	Progressor	Non-Progressor			
1	ACAGATACTGCC	0.37	0.43	0.29	1.91	1.16-3.15	*0.012*
2	CTGGGCATTATG	0.22	0.21	0.20	0.97	0.54-1.74	*0.91*
3	ATAGATTCCGTG	0.13	0.09	0.17	0.45	0.21-0.98	*0.044*
4	ATGAGTTCCGTG	0.11	0.09	0.14	0.53	0.26-1.09	*0.084*
5	CTGAGTTCCGTG	0.04	0.05	0.03	1.48	0.45-4.84	*0.521*
6	ATAGATACTGCC	0.02	0.02	0.02	1.21	0.21-7.05	*0.835*
7	ATAGGCATTATG	0.02	0.03	0.01	4.51	0.50-40.26	*0.178*
8	CTAGATTCTATG	0.02	0.01	0.03	0.45	0.08-2.67	*0.381*
9	ACAGATACTGCG	0.01	0.01	0.01	1.25	0.11-14.5	*0.86*

*SNP order in haplotypes: rs315931/rs4251961/rs2637988/rs3181052/rs1794066/rs419598/rs380092/rs579543/rs315952/rs9005/rs315943/rs1374281

Table 6

Best 2 to 6 SNP models relative to radiographic knee OA progression

Number of SNPs	SNP order within haplotypes	Haplotype	Freq	Odds ratio	*P value* *	q value (False Discovery Rate)
6	RS3181052|RS1794066|RS419598|RS315952|RS9005|RS315943	GATTGC	0.41	1.96	*0.0086*	0.0432
		GGCTAT	0.26	1.16	*0.6130*	0.6130
		GATTAT	0.04	0.08	*0.0233*	0.0583
		AGTCGT	0.12	0.65	*0.1610*	0.2150
		GATCGT	0.15	0.6	*0.1720*	0.2150
	Omnibus p value =0.001066					
5	RS3181052|RS1794066|RS419598|RS9005|RS315943	GATGC	0.41	2.04	*0.0061*	0.0304
		GGCAT	0.26	1.16	*0.6130*	0.6130
		GATAT	0.04	0.09	*0.0232*	0.0580
		AGTGT	0.12	0.65	*0.1610*	0.2163
		GATGT	0.15	0.6	*0.1730*	0.2163
	Omnibus p value =0.0009193					
4	RS3181052|RS419598|RS9005|RS315943	GTGC	0.41	2.04	*0.0059*	0.0297
		GCAT	0.26	1.16	*0.6130*	0.6130
		GTAT	0.04	0.08	*0.0233*	0.0583
		ATGT	0.12	0.66	*0.1850*	0.2313
		GTGT	0.15	0.55	*0.1240*	0.2067
	Omnibus p value =0.0008631					
3	RS419598|RS9005|RS315943	TGC	0.41	2.05	*0.0059*	0.0235
		CAT	0.26	1.16	*0.6130*	0.6130
		TAT	0.04	0.09	*0.0233*	0.0466
		TGT	0.28	0.57	*0.0355*	0.0473
	Omnibus p value =0.0003149					
2	RS419598|RS315943	TC	0.42	2.04	*0.0061*	0.0091
		CT	0.27	1.11	*0.7350*	0.7350
		TT	0.31	0.44	*0.0019*	0.0058
	Omnibus p value =0.002012					

*Adjusted for age, gender and BMI

Table 7

Frequencies of the 3-SNP (rs419598/rs9005/rs315943) IL1RN haplotypes in radiographic progressors and non-progressors, as measured by change in KL score and by categorical joint space narrowing (JSN) scores

Haplotype rs419598/rs9005/rs315943	Freq in KL progressors	Freq in KL non-progressors	Unadjusted p-value	Freq in JSN progressors	Freq in JSN non-progressors	Unadjusted p-value
CAT	0.28	0.24	*0.534*	0.27	0.30	*0.74*
TGC	0.48	0.32	*0.006*	0.48	0.30	*0.015*
TGT	0.25	0.36	*0.041*	0.24	0.36	*0.088*
TAT	0.01	0.08	*0.017*	0.01	0.05	*0.12*
Omnibus			0.0003			*0.044*

Table 8

Conditional analysis of IL1RN haplotypes

	p value
12 SNP Haplotype omnibus model	**0.023**
SNP Conditioned	**p-value after SNP Conditioning** [1]
RS315931	0.009
RS4251961	0.245
RS2637988	0.017
RS3181052	0.021
RS1794066	0.017
RS419598	0.009
RS380092	0.250
RS579543	0.009
RS315952	0.250
RS9005	0.009
RS315943	0.245
RS1374281	0.245

[1] p-value for haplotype association with progression after conditioning of the single indicated SNP in the model