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Association of erosive hand osteoarthritis with a single nucleotide polymorphism on the gene encoding interleukin-1 beta

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Summary

Objective: Certain forms of primary osteoarthritis (OA), particularly those affecting hand joints, have a genetic component. Recent studies have shown suggestive evidence that hand and knee OA are linked with the interleukin-1 (IL-1) region on human chromosome 2q. This study was undertaken to assess the association of primary OA of the hand (hand OA) with IL-1 region markers.

Methods: Sixty-eight US Caucasoid cases and 51 US Caucasoid controls aged 60 years or older were recruited from the Mid-Atlantic region of the United States. Hand OA was classified by American College of Rheumatology (ACR) Clinical Criteria, and cases were subjected to radiographic examination for subgrouping. Genotyping was done for seven previously described single nucleotide polymorphisms (SNPs) of genes for IL-1α (encoded by IL1A), IL-1β (IL1B), and the IL-1 receptor antagonist (IL1RN), as well as an IL1RN variable number of tandem repeat (VNTR) marker. Six microsatellite markers on other chromosomes (null loci) were also typed.

Results: The IL1B 5810 G>A SNP genotypes marker were not in Hardy–Weinberg equilibrium ($p<0.05$ in both non-erosive and erosive hand OA subgroups). Statistically significant association with the IL1B 5810 AA genotype was found in the erosive hand OA subgroup (relative risk 3.8, $p=0.007$). This IL1B 5810 AA genotype association was also significant between erosive and non-erosive hand OA subjects (relative risk 4.01, $p=0.008$). As expected, significant linkage disequilibrium was present between IL1B 5810 SNP and IL1A (–)889 SNP, other IL1B SNPs, and the nearest IL1RN SNP examined. The IL1B 5810A allele occurs most frequently on haplotypes with the SNP alleles IL1B 1423C, IL1B 1903T, IL1B 5887C, and IL1A (–)889C. Genotypes at null loci failed to show evidence suggesting population stratification that might account for spurious association.

Conclusion: Statistical evidence shows association between erosive hand OA and a genomic region containing the IL1B 5810 SNP in a US Caucasoid population. This supports a potential role for IL-1 in the pathogenesis of a severe phenotype of hand OA.

Key words: Osteoarthritis, Hand, Genetic, Interleukin-1.

Introduction

Osteoarthritis (OA), a chronic disorder characterized by pain, stiffness, and reduced range of motion of the affected joints, has a major heritable basis. Familial aggregation is present[1], and the intraclass correlation coefficient in monozygotic twins is higher than in dizygotic twins, 0.64 vs 0.38[2]. However, the heritable OA component is genetically complex and does not follow a strictly Mendelian pattern. One such complexity is incomplete penetrance, which is influenced by sex. OA is generally a late-onset disease, and OA prevalence increases with age. OA prevalence in men and women is not remarkably different in the fourth and fifth decades, but women have a higher prevalence in

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older cohorts. In the population aged 65 and older, 38% of men and 62% of women had radiographic evidence of hand OA[3]. Another complexity is genetic heterogeneity. For genetic studies, recognizing important variations in clinical picture that subdivide an illness into genetically more homogenous subsets is important. OA involving the distal interphalangeal (DIP) joints of the hands (Heberden's nodes) has been described as a distinctly heritable subset[4,5]. Genomic screens have been undertaken for primary OA[6–8]; genomic intervals on human chromosomes 2, 4, and 16 showed suggestive linkage signals in more than one scan[9].

Recent genetic studies point specifically toward the interleukin-1 (IL-1) region. A genome-wide screen by Leppavuori *et al.* demonstrated suggestive evidence of linkage of hand OA to the IL-1 region[6]. Loughlin *et al.* noted a case-control association of IL-1 region markers with end-stage knee OA; although one would expect many subjects to also have hand OA, the published report did not include stratification by hand OA presence[10]. Given the suggestive linkage and association evidence of certain primary OA subgroups with the IL-1 region, we sought to examine association in a population with hand OA.

Subjects and methods

HUMAN SUBJECTS

Control subjects were identified and recruited from outpatient clinics at the McGuire Veterans Affairs Medical Center (VAMC), Richmond, VA, and rheumatology offices. Eligible participants were unrelated individuals aged 60 years and older who did not have evidence of hand OA, using the American College of Rheumatology (ACR) Clinical Criteria[11]. After informed consent, a rheumatologist (AGS or TPSR) examined each subject's hands. Exclusion criteria were any joint examination evidence of primary hand OA: more than one Heberden's or Bouchard's node, bilateral first carpometacarpal (CMC) disease, any hand deformity, or the presence of any known inflammatory arthropathy or clinical evidence of joint inflammation of the hands. Traumatic injury may lead to hard tissue enlargement and deformity of a single interphalangeal (IP) joint. Participating subjects underwent peripheral venous phlebotomy for DNA purification and filled out a questionnaire about demographic information and current medications. Because all but two OA subjects listed ancestry as Caucasian, only Caucasoid controls were included in this analysis (Table II).

Hand OA subjects

Unrelated subjects were recruited from primary care and rheumatology practices, targeted mailings to prospective subjects, and a newspaper advertisement campaign. After informed consent, subjects were evaluated at the VAMC or at a participating site (see Acknowledgements). At the study visit, the evaluation included a postero-anterior radiograph and digital photograph of each hand, venous phlebotomy, a bone mineral density scan of the lumbar spine and hip (and distal forearm in a subset of subjects) using dual energy X-ray absorptiometry (DEXA), and a targeted rheumatology history and physical examination (TPSR or AGS). The clinical evaluation addressed symptoms and signs referable to OA, potential causes of secondary OA, medications, cigarette smoking history, and examination of OA joint distribution. It also included an assessment of joint loading by recording of work history, as well as ethnic and demographic information. Hand OA was classified utilizing the ACR Classification for Clinical Hand OA[11]. In particular, subjects were required to have physical examination evidence of hard tissue enlargement and/or deformity in three or more index hand joints as listed in the criteria (first CMC, second and third proximal (PIP) and DIP joints of each hand). Subjects with OA of the first CMC but no other involved hand joints were included if bilateral disease was present. Subjects participated in the study on one day; greater than 90% of the examinations were performed by one rheumatologist (AGS). Therefore, no intra-rater or inter-rater evaluations were performed for the examination.

One musculoskeletal radiologist (DD) evaluated hand radiographs according to the Kellgren–Lawrence (K–L) scale[12]. For this analysis, radiographic hand OA was defined as three or more index joints (as in the ACR Clinical Criteria) with K–L score of 2–4, as well as the absence of soft-tissue swelling in two or more metacarpophalangeal (MCP) joints. A radiographic hand OA classification also required the absence of erosions in the MCPs, carpal joints, or radio-carpal joint. Intra-rater agreement for the overall diagnosis of radiographic hand OA was acceptable (κ=0.76). Additional, intra-rater agreement for K–L scores of either '0–1', '2', or '3–4' in individual joints was good (median weighted κ=0.78, range 0.65–0.88). Subjects were excluded for a previous diagnosis of inflammatory arthritis, psoriasis, or calcium pyrophosphate deposition disease (CPPD). Hand radiographs were also used to subdivide the hand OA subjects into erosive and non-erosive subgroups. Erosive OA was defined by previously described criteria, namely, radiographic central erosions and/or ankylosis in the IP joints of the hand in at least three digits[13], associated with joint-space narrowing, subchondral sclerosis, and/or osteophytes (Fig. 1). Subjects with OA in other joints typically involved by primary OA, including knee, hip, and cervico-lumbar spine were included, as long as ACR Clinical Criteria for Hand OA were met.

The protocol and all study procedures were approved and monitored by a local Institutional Review Board.

GENETIC TYPING

DNA was purified from EDTA-anticoagulated venous blood using a salting-out method (PureGene, Gentra, Minneapolis, MN).

IL1RN VARIABLE NUMBER OF TANDEM REPEAT (VNTR) GENOTYPING

The IL1RN VNTR is based on a tandemly repeating 86 bp subunit in the second intron. PCR products[14] were derived by amplication with 50 ng DNA template, 5 μM of each primer, 2 mM $MgCl_2$, 0.5 mM dNTPs (Applied Biosystems, Inc., Foster City, CA), and AmpliTaq Gold® DNA polymerase (Applied Biosystems). Annealing temperature was 59°C. PCR products were then subjected to electrophoretic separation in 1.5% Metaphor agarose gel in 1× TBE (Cambrex, Rockland ME), stained with ethidium bromide, and visualized under ultraviolet light. Forward and reverse primers were as follows: 5′ CTCAGCAACACTCC TAT 3′; 5′ TCCTGGTCTGCAGGTAA 3′. Alleles are conventionally defined as follows: Allele 1 (412 bp, representing four repeats); Allele 2 (240 bp, two repeats); Allele 3 (498 bp, five repeats); Allele 4 (326 bp, three repeats)[14].

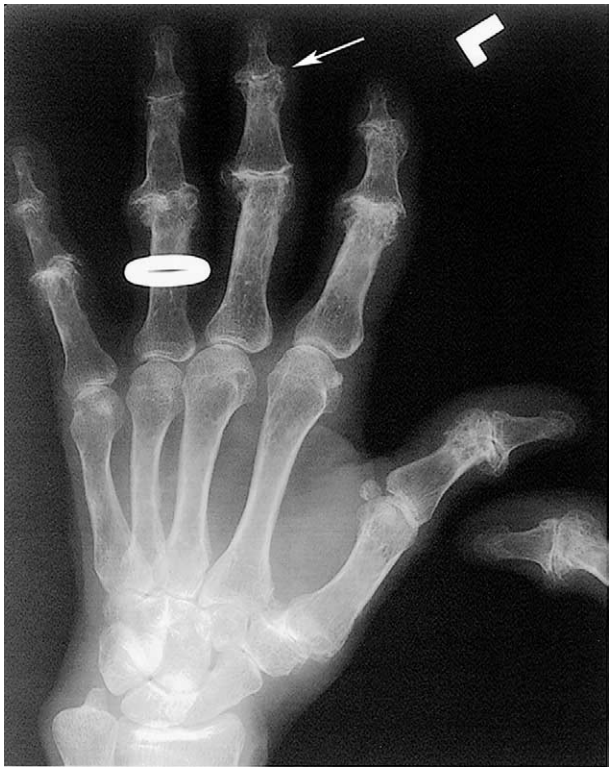

Fig. 1. Radiographic features of erosive OA. Notable for central, cartilage-based, 'gull-wing' erosions (white arrow, typical example). Multiple IP joints with asymmetric joint-space narrowing, along with subchondral sclerosis, and osteophytes. There is a relative sparing of the MCP joints.

GENOTYPING OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS)

SNP genotyping was performed using the TaqMan® method for allele-specific detection (Applied Biosystems). The TaqMan® genotyping method involves a real-time PCR amplification with fluorescence detection[15]. TaqMan® probes were designed using Primer Express® version 1.5 (Applied Biosystems). The probes were labeled at the 5′ end with FAM® for each major allele and with TET® for each minor allele and at the 3′ end with TAMRA®. The experiments were performed in the ABI Prism® 7900 Sequence Detection System (Applied Biosystems) using the TaqMan® Universal PCR Master Mix (Applied Biosystems), along with 20 ng genomic DNA template (10 ng/μl). All samples were tested in duplicate under conditions recommended by the manufacturer, with an annealing temperature of 60°C. The PCR reactions and synthesis of the probes and primers (Table I) were performed in the Nucleic Acids Research Facilities at Virginia Commonwealth University (VCU), Richmond, VA. One internal control sample was repeatedly tested with each grouping of samples; two additional internal control samples were tested with one additional batch. No SNP typing errors were found.

GENOTYPING OF NULL LOCI (POLYMORPHIC MICROSATELLITE MARKERS DISTANT FROM HUMAN CHROMOSOME 2Q)

The AmpFLSTR® COfiler™ PCR Amplification Kit (Applied Biosystems) was used to amplify the repeat regions of six microsatellites (D3S1358, D16S539, THO1, TPOX, CSF1PO, D7S820) (mean heterozygosity 0.75±SD 0.06, range 0.64–0.81) and also the amelogenin gene for sex identification. Ten nanograms of genomic DNA in 8 μl was combined with 12 μl AmpFLSTR® COfiler™ PCR master mix (8.4 μl PCR reaction mix; 0.4 μl Amplitaq Gold DNA polymerase; 4.4 μl primer set) and then subjected to PCR amplification with annealing temperature 59°C. The PCR products were subjected to electrophoretic separation in 5% polyacrylamide gel using an ABI Prism® 377 DNA Sequencer. GeneScan Analysis® 3.1 and Genotyper® 2.1 Software were used for data analysis. Reactions and genotyping analyses were performed in the Nucleic Acids Research Facilities at VCU.

STATISTICS

To assess for mistyping (and as noted under SNP typing), we analyzed an internal control sample with every batch of samples processed ($n=5$), and two additional samples were repeated in an additional analysis. We found

Table I
Primers and probes for IL-1 region SNPs

Gene	Location	Polymorphism	Primers/probes	Reference
IL 1A	Promoter	−889C>T†	5′CACAGGAATTATAAAAGCTGAGAAATTC3′; 5′GGAGAAAGGAAGGCATGGATT3′/ 5′CCAGGCAACA**C**/**T**CATTGAAGGCTCATATG3′	52
IL 1B	Exon 5	5887C>T†	5′GGCCTGCCCTTCTGATTTTATA3′; 5′TCGTGCACATAAGCCTCGTTA3′/ 5′TTCAGAACCTATCTTCTT**C**/**T**GACACATGG3′	23
IL 1B	Intron 4	5810G>A†	5′CAGGTGTCCTCCAAGAAATCAAA3′ 5′TGTGGAGCACATGTTGTTTAGGTA3′/ 5′TTGCC**G**/**A**CCTCGCCTCACGAG3′	23
IL 1B	Promoter	1903T>C†	5′CCCTTTCCTTTAACTTGATTGTGAAAT3′; 5′AGGTTTGGTATCTGCCAGTTTCTC3′/ 5′CCTCGCTGTTTTTTAT**A**/**G**GCTTTCAAAAGCA3′	23
IL 1B	Promoter	1423C>T†	5′CCTCAGAGGCTCCTGCAATT3′; 5′TGAGGGTGTGGGTCTCTACCTT3′/ 5′TTCTCTGCCTC**G**/**A**GGAGCTCTCTGT3′	53
IL 1RN	Exon 2	8006 T>C	5′CTGAGTCCTTTTCCTTTTCAGAATCT3′/5′CAACCACTCACCTTCTAAATTGACA3′/ 5′CAACCAACTAGTTGC**T**/**C**GGATACTTGCAAG3′	54
IL 1RN	Exon 4	11100 T>C	5′CCTGAGCGAGAACAGAAAGCA3′; 5′CAGGCGGCAGACTCAAAACT3′/ 5′ATCCGCTCAGACAG**T**/**C**GGCCCCA3′	23

Top row for each SNP represent forward/reverse amplimers. Second row represents TaqMan probe, with alternative bases in bold font representing the polymorphism.

†Seattle SNP designations IL1A5138, IL1B5277, IL1B5200, IL1B1274, and IL1B0794, respectively[22].

Table II
Subject characteristics

	Erosive hand OA	Non-erosive hand OA	Controls
Number	26	42	51
Sex	21 females, 5 males	33 females, 9 males	6 females, 45 males
Age (years)			
Male (mean±SD)	73±6.6	69±6.4	72±6.7
Female (mean±SD)	71±7.1†	65±10.8	69±10.0
Joint count‡§			
Radiograph‖	15.9±4.1	7.5±5.4	N/A
Nodes	15.0±4.0	9.6±4.2	N/A
Deformities¶	5.7±3.7	1.3±1.3	N/A

†p=0.031 female erosive OA vs female non-erosive OA.
‡First CMC, all IP joints bilaterally, maximum n=20.
§p<0.0001 for all comparisons, erosive vs non-erosive groups.
‖Number of joints with a K–L radiographic score of 2, 3, or 4.
¶Horizontal or fixed flexion deformities.

identical genotypes at every retyping at each IL1-region marker. Heterozygosity and Hardy–Weinberg equilibrium were assessed using linkage utility software[16]. Case-control association studies may sometimes be subject to interference by minor deviations from Hardy–Weinberg equilibrium in controls, and thus relative risks may vary among studies. To address this possibility, allele and genotype frequencies, as well as genotype relative-risk ratio, were analyzed using the Lathrop method[17]; this adjusts control genotype frequencies to conform to Hardy–Weinberg equilibrium. Regarding correction for multiple comparisons, the IL-1 region markers represent the first OA markers we typed in a genomic region with some previously reported linkage evidence and substantial biological evidence supporting a candidate gene. Several typed markers are in linkage disequilibrium, so testing one marker in tight linkage disequilibrium with another is nearly the same as testing the second. Thus, a strict Bonferroni adjustment by numbers of markers is not reasonable. For this reason, the p-values reported are not adjusted for number of comparisons; instead, a conservative correction would allow for multiplying our reported p-values by the three loci represented. Linkage disequilibrium was analyzed using an expectation-maximization algorithm (the GOLD software procedure ldmax)[18]. As is true with other late-onset diseases, OA subjects typically do not have parents available for study. For that reason, one cannot easily establish haplotypes by family studies but must reconstruct haplotypes from population genotype data. We used a Markov-chain method for haplotype assignments (PHASE[19]). The validity of case-control studies for disease association may sometimes be threatened by population stratification. We used the stratification χ^2 statistic to test empirically for this potential problem[20]. We also examined the best-fit probabilities with a model-based clustering method among models assuming one to five clusters (STRUCTURE[21]). This method is based on fitting models postulating one or more groups to actual null locus genotypes; if the best-fitting model posits more than one empirical cluster, it is consistent with population stratification.

Results

PHENOTYPE

Cases and controls were similar in age and of US Caucasoid ethnicity. Cases were 79.4% female; controls were 88.2% male (Table II). Sixty-one of the 68 cases identified by ACR Clinical Criteria also fulfilled K–L radiographic criteria. When the subgroup with radiographically erosive OA was compared with non-erosive OA, the female erosive hand OA subgroup was slightly, though significantly older (p=0.031). In general, the erosive subgroup had more extensive hand OA, whether measured by radiographic joint count, number of nodes, or number of deformities (Table II).

ABSENCE OF POPULATION STRATIFICATION

Allele frequencies for six null loci matched those for the US Caucasian population (according to manufacturer's literature). The null loci showed no significant evidence of population stratification across groups defined by presence or absence of ACR Clinical Criteria for Hand OA (stratification χ^2=48.0, df=38, p=0.13). Modeling null locus genotype frequencies for one to five clusters showed that we were unable to reject the hypothesis that only one cluster (population) existed among the subjects. Thus, the analyses failed to show population structure or admixture.

IL-1 REGION MARKERS

Genotype frequencies for all groups are presented in Table III. Control frequencies matched those reported in previous studies for US subjects of European descent[22]. Among all SNP markers other than (IL1B) 5810 in OA subjects, there was no departure from Hardy–Weinberg equilibrium in either OA or control groups. The sole exception to Hardy–Weinberg equilibrium was the IL1B 5810 SNP in erosive and non-erosive OA subjects (among all hand OA, χ^2 11.994, p=0.000537; among erosive OA subgroup, χ^2 3.846, p=0.05; in the non-erosive hand OA subgroup, χ^2 7.433, p=0.006419). As with all other SNP assays, these samples were typed at this SNP in duplicate in parallel with controls. We had no basis to suspect selective mistyping of OA subjects at this one marker and found identical genotypes with retyping in three subjects.

To compare genotype frequencies at each IL1-region marker, we estimated genotype-associated risks after adjusting control frequencies to achieve Hardy–Weinberg equilibrium. Statistically significant results are given in Table IV. IL1B 5810 genotype AA was associated with a modestly elevated risk for all hand OA subjects compared

Table III
*IL-1 region genotype frequencies among cases and controls**

Locus	Genotype	Non-erosive OA subjects (*n*=42)	Erosive OA subjects (*n*=26)	Control Subjects (*n*=51)
IL1A-889 C>T	CC	27 (27.5)	8 (9.8)	28 (28.3)
	CT	14 (13.0)	16 (12.3)	20 (19.4)
	TT	1 (1.5)	2 (3.8)	3 (3.3)
IL1B 5887 C>T	CC	30 (30.0)	15 (14.6)	33 (32.2)
	CT	11 (11.0)	9 (9.8)	15 (16.7)
	TT	1 (1.0)	2 (1.6)	3 (2.2)
IL1B 5810 G>A	GG	22 (18.0)	9 (6.5)	21 (21.4)
	GA	11 (19.0)	8 (13.0)	24 (23.3)
	AA	9 (5.0)	9 (6.5)	6 (6.4)
IL1B 1903 T>C	TT	15 (14.3)	17 (15.4)	17 (18.2)
	TC	19 (20.4)	6 (9.2)	27 (24.5)
	CC	8 (7.3)	3 (1.4)	7 (8.2)
IL1B 1423 C>T	CC	15 (14.3)	17 (15.4)	17 (18.2)
	CT	19 (20.4)	6 (9.2)	27 (24.5)
	TT	8 (7.3)	3 (1.4)	7 (8.2)
IL1RN 8006 T>C	TT	17 (15.5)	19 (17.8)	23 (24.0)
	TC	17 (20.0)	5 (7.4)	24 (22.0)
	CC	8 (6.5)	2 (0.8)	4 (5.0)
IL1RN VNTR†	1,1	16 (14.3)	17 (16.2)	22 (22.4)
	1,2	16 (19.3)	5 (7.1)	22 (20.8)
	2,2	8 (6.5)	2 (0.8)	4 (4.8)
	1,3	1 (1.2)	2 (1.6)	1 (1.3)
	2,3	1 (0.8)	0 (0.3)	1 (0.6)
IL1RN 11100 T>C	TT	27 (26.7)	11 (12.1)	34 (33.0)
	TC	13 (13.6)	14 (11.1)	14 (16.1)
	CC	2 (1.7)	1 (2.5)	3 (2.0)

*Values in parentheses represent genotype frequencies expected under the assumption of Hardy–Weinberg equilibrium.
†*n*=50 for control VNTR (insufficient DNA available for one sample).

with controls. However, in the erosive OA subgroup, the IL1B 5810 AA genotype association was present, when comparing with either controls or the non-erosive hand OA subjects. In comparing the erosive hand OA with the other OA subjects, as well as with the controls, other significant genotypic associations were noted.

LINKAGE DISEQUILIBRIUM AMONG IL-1 REGION MARKERS

Utilizing an expectation-maximization method, we assessed for linkage disequilibrium (Fig. 2). Significant linkage disequilibrium extends from the IL1B 5810 SNP to flanking IL1B SNP markers as well as centromeric to IL1A (–)889 and telomeric to IL1RN 8006. The genomic interval in linkage disequilibrium with the IL1B 5810 SNP (IL1A to IL1RN) is over 300 kb long.

IL-1 REGION HAPLOTYPES

Statistical reconstruction of haplotypes using population genotype data was performed. Among the subjects, the most common haplotype bearing the IL1B 5810 A allele occurred with the major alleles at the other SNP markers: IL1A (–)889C, IL1B 1423C, and IL1B 5887T. This haplotype represented 81 haplotypes among 240 total haplotypes for all cases and controls, 49 in cases and 32 in controls. Two other haplotypes bearing the IL1B 5810A allele were found, accounting for 11 haplotypes in total, seven in cases, four in controls. While no significant association of IL1B 5810A-bearing haplotype with OA was noted, the numbers examined did not allow adequate power to demonstrate this difference.

Table IV
Genotype risks for hand OA and erosive hand OA for IL-1 region markers

Group/marker	Genotype	Risk	*p*-value*
All OA vs. controls			
IL1B 5810 G>A	GG	1.14	0.713
	GA	0.49	0.012 (0.036)
	AA	2.53	0.023 (0.069)
Erosive OA vs. controls			
IL1B 1423 C>T	CC	3.31	0.011 (0.033)
	CT	0.34	0.016 (0.048)
	TT	0.77	0.674
IL1B 5810 G>A	GG	0.75	0.546
	GA	0.55	0.150
	AA	3.82	0.007 (0.021)
IL1RN 8006 T>C	TT	2.92	0.028 (0.084)
	TC	0.34	0.024 (0.072)
	CC	0.94	0.922
IL1RN 11100 T>C	TT	0.41	0.056
	TC	2.52	0.035 (0.105)
	CC	1.47	0.647
Erosive OA vs non-erosive hand OA			
IL1A-889 C>T	CC	0.24	0.005 (0.015)
	CT	3.52	0.006 (0.018)
	TT	2.71	0.195
IL1B 1423 C>T	CC	3.57	0.008 (0.024)
	CT	0.34	0.013 (0.039)
	TT	0.71	0.581
IL1B 5810 G>A	GG	0.72	0.502
	GA	0.56	0.168
	AA	4.01	0.008 (0.024)
IL1RN 8006 T>C	TT	4.45	0.003 (0.009)
	TC	0.28	0.007 (0.021)
	CC	0.56	0.400

*Values in parentheses represent significant p-values corrected for multiple comparisons.

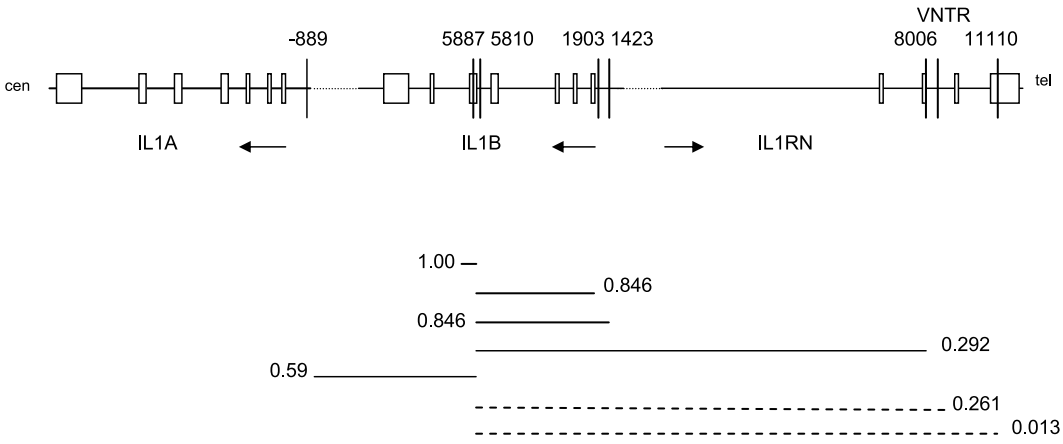

Fig. 2. IL-1 region linkage disequilibrium. Schematic representation of selected IL-1 region loci on human chromosome 2q, drawn from the draft sequence, June 2002 freeze of the Human Genome Project (http://www.genome.ucsc.edu). Distances between loci are not to scale; that between IL1A and IL1B is approximately 52 kb, between IL1B and IL1RN is nearly 300 kb. Individual SNP and VNTR markers are represented as vertical lines, exons are boxes. Each arrow represents the direction of transcription for the individual locus. Numbers and lines below map represent disequilibrium statistics: the D′ value (pairwise standardized disequilibrium coefficient) is shown at the end of each line; statistically significant linkage disequilibrium ($p<0.05$) between IL1B 5810 SNP and other nearby markers and IL1B 5810 SNP is depicted with solid lines and $p>0.05$ is represented by dashed lines.

Discussion

Taken as a whole, the data support the notion that the IL-1 region contains a candidate gene for hand OA, particularly in the erosive OA subset. We considered potential problems related to sex differences, phenotype classification, and spurious associations. Because cases were predominantly female and controls were largely male, one might consider the sex influence on hand OA penetrance[3]. The control allelic frequencies at the IL1B 5810 marker were similar to those in previous studies[22,23]. The direction of bias from the sex difference is likely to reduce differences in genotype frequencies of OA candidate genes. At the subject age in this study, the incompletely penetrant fraction, that is, those subjects who bear a disease-associated genotype without the clinical manifestation, will be larger in males than in females. This means, because of diminished male penetrance, the disease-associated markers would be more frequent in the largely male controls than would be true if there was complete penetrance. Thus, the sex difference between cases and controls represents a conservative bias, one that would tend to hide rather than exaggerate a true difference in genotype frequencies. This interpretation is bolstered by the IL1B 5810 AA genotype difference between erosive and non-erosive OA, both largely female groups.

One might consider also the influence of using ACR Clinical Criteria for Hand OA to choose whether to do a hand radiograph. Control individuals were examined and classified as lacking ACR Clinical Criteria but not subjected to a radiograph. A negative examination typically indicates low prevalence of radiographic OA. In the absence of hand complaints, the clinical observation of no hard tissue enlargement has a moderate to high negative predictive value for radiographic OA[24]. Additionally, the absence of a Heberden's node has a good negative predictive value for radiographic osteophyte in the corresponding joint[25]. In our study, the cases classified as having ACR Clinical Criteria for Hand OA had some hand joints that were clinically normal. These joints with normal examination were unlikely to show radiographic OA features (median negative predictive value was 82.58 for IP joints with a range 68.29–100.00 (data not shown)). Yet another consideration is spurious associations due to population stratification, such as ethnic admixture, consanguinity, or other undetected genetic influences. Our analyses of null loci do not support the assertion that our recruitment strategy led to population stratification, so that cause of spurious association seems unlikely. Additionally, we have reasoned that it would not be appropriate in this study to adjust results for every marker comparison; however, even if one utilized the conservative Bonferroni method to adjust for three loci this study's major association still appears significant. Finally, given linkage disequilibrium between the IL1B 5887 major allele and IL1B 5810 minor allele, one might wonder why association of the IL1B 5887 with erosive hand OA was not found. Indeed, the high frequency of the IL1B 5887 major allele (approximately 0.80) suggests that the number of subjects examined did not provide sufficient power to detect an association at this locus. Therefore, the statistical support for an IL-1 region candidate gene seems believable.

An IL-1 effect in OA pathogenesis seems similarly credible. Over two decades ago, Saklatvala and Dingle described a polypeptide substance catabolin that stimulated chondrocytes to resorb their surrounding matrix without the aid of extrinsic enzymes. From various biological activities, this catabolin was later found to be IL-1[26]. With availability of cloned and purified IL-1, many research groups have found that IL-1 affects cartilage. Local injection of IL-1 into rabbit knee joints induces a neutrophilic synovial fluid infiltrate and augments proteoglycan catabolism. *In vitro*, IL-1 causes cartilage breakdown and stimulates arachidonic acid release from cell membranes and subsequent production of prostanoids and eicosanoids[27]. Thus, because IL-1 stimulates cartilage degradation and induces production of other substances that also serve to foster cartilage breakdown, an IL-1-related locus represents a biologically reasonable candidate gene for OA. The IL-1 network may be important in OA progression and thus severity. Pelletier *et al.*[28] have noted that IL1B is the predominant cytokine involved in the stimulation of metalloproteinase production within OA synovium. Furthermore, Martel-Pelletier and colleagues have demonstrated that OA

chondrocytes elaborated significantly more metalloproteinases with *in vitro* IL-1 stimulation than do chondrocytes from normal controls[29]. Exposing cartilage explants, either OA or normal, to IL-1 causes accelerated proteoglycan release[30]. Also, Martel-Pelletier *et al.*[29] have found that the IL-1 receptor type 1 density on the surface of chondrocytes from OA-affected subjects was twice that present on the chondrocytes from normal controls. Taken as a whole, this body of evidence regarding IL-1 effects on cartilage suggests that OA may stem from a self-destructive process involving IL-1 and chondrocytes. For these reasons, this study's statistical support for an IL-1 region candidate gene in OA is biologically plausible.

Exactly which gene is the candidate is unknown. The SNP marker most closely tied to erosive OA in this study was IL1B 5810. The IL1B 5810 SNP's location in the fourth intron does not suggest an obvious effect on mRNA splicing; while the IL1B 5810 SNP could influence mRNA stability, it is more likely a haplotype marker for a more important polymorphism in linkage disequilibrium. The region in linkage disequilibrium with this SNP is fairly large, several hundred kilobases, extending telomerically to IL1RN and centromerically at least to IL1A. Some genetic polymorphisms of the IL-1 region have functional differences. IL1RN VNTR allele 2 is associated with several inflammatory diseases, including inflammatory bowel diseases, psoriasis, and multiple sclerosis[31]; the influence of IL1RN VNTR allele 2 on protein levels of IL-1Ra has been examined, but various studies have conflicting results[32–37]. The IL1RN VNTR allele 2 is associated with increased *in vitro* IL1B production from stimulated mononuclear cells[38]. In addition, the TT genotype of IL1B 5887 C>T SNP (recognized as presence/absence of a *Taq*I restriction site polymorphism) correlates with significantly higher *in vitro* IL1B secretion[39]. Finally, the TT genotype of the IL1A-889 C>T SNP has been associated with an increase in IL1-α production by LPS-stimulated peripheral blood mononuclear cells from healthy volunteers[40]. Thus, genetic polymorphisms exist, some of which may be of functional significance in some cell lineages, but the influence on cells important to OA (i.e., chondrocytes) is unknown.

Erosive hand OA is likely a more severe hand OA phenotype, and this case-control study seems to support genetic heterogeneity. Whether erosive OA stems from a different etiology from non-erosive, nodal OA, or whether it is simply a more severe disease phenotype is unknown. Indeed, erosive OA, initially termed interphalangeal OA by Crain[41], has been described as a distinct clinical subset of hand OA. In addition to typical nodal features, subjects have recurrent inflammatory episodes, often present even early in the disease course, with deformity and ankylosis potentially ensuing[42]. A marked female predominance has been noted[43]. Furthermore, two studies have examined specific genetic markers. In one study, the MZ genotype of α1-antitrypsin was significantly associated with erosive OA compared with non-erosive OA or with controls[44]. In another more recent study from Argentina, no significant differences were noted in frequencies of a matrillin-1 gene polymorphism compared with controls, either for the nodal or erosive phenotype[45]. In view of the role IL-1 plays in rheumatoid arthritis, association of IL-1 region markers with erosive OA makes sense. Indeed, IL-1 has been shown to be an important mediator of erosions in rheumatoid arthritis[46], and studies have noted a suggestive linkage of a severe rheumatoid arthritis phenotype to the IL-1 region[47,48]. Horai *et al.*[49] highlighted the importance of IL-1 network regulation by describing the IL1RN knockout mouse phenotype to include an erosive arthritis. Finally, treatment of rheumatoid arthritis with recombinant human IL-1Ra results in amelioration of clinical symptoms and reduction of erosions[50]. Therefore, IL-1 may be associated with the more severe spectrum of OA phenotype, as well as more severe rheumatoid arthritis. One may speculate that the character and distribution of IL-1-related erosions may be partly explained by the specific cell lineage important for the disease (i.e., rheumatoid arthritis with synovial tissue and mononuclear cells, and OA with chondrocytes).

In conclusion, we note a significant association between markers on the IL-1 region and erosive hand OA. Because this case-control association is supported by a relatively low *p*-value generated by the first set of candidate-gene markers typed, and the associated genomic region bears not only linkage evidence but also biological plausibility for OA, we find the support for an IL-1 region candidate gene in OA persuasive. However, as with other case-control studies, this statistical observation must be reproduced to be regarded as true. If the finding is reproducible, one may do further genetic studies to pinpoint a disease-related gene. Studies to establish mechanism might include assays of chondrocyte and nonchondrocyte production of IL-1 region proteins and correlation between protein levels and IL-1 region genetic polymorphisms in diseased and control individuals. In the future, clinicians might examine specific IL1-β inhibitors[51] in subjects with a genetic predisposition to erosive hand OA.

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