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A Broad Analysis of *IL1* Polymorphism and Rheumatoid Arthritis

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Objective. **It has been suggested that polymorphisms in *IL1* are correlated with severe and/or erosive rheumatoid arthritis (RA), but the implicated alleles have differed among studies. The aim of this study was to perform a broad and well-powered search for association between allelic polymorphism in *IL1A* and *IL1B* and the susceptibility to or severity of RA.**

Methods. **Key coding and regulatory regions in *IL1A* and *IL1B* were sequenced in 24 patients with RA, revealing 4 novel single-nucleotide polymorphisms (SNPs) in *IL1B*. These and a comprehensive set of 24 SNPs tagging most of the underlying genetic diversity were genotyped in 3 independent RA case–control sample sets and 1 longitudinal RA cohort, totaling 3,561 patients and 3,062 control subjects.**

Results. **No fully significant associations were observed. Analysis of the discovery case–control sample sets indicated a potential association of *IL1B* promoter region SNPs with susceptibility to RA (for RA3/A, odds ratio [OR] 1.27, $P = 0.0021$) or with the incidence of radiographic erosions (for RA4/C, OR 1.56, $P = 0.036$), but these findings were not replicated in independent case–control samples. No association with rheumatoid factor, anti–cyclic citrullinated peptide, or the Disease Activity Score in 28 joints was found. None of the associations previously observed in other studies were replicated here.**

Conclusion. **In spite of a broad and highly powered study, we observed no robust, reproducible association between *IL1A/B* variants and the susceptibility to or severity of RA in white individuals of European descent. Our results provide evidence that, in the majority of cases, polymorphism in *IL1A* and *IL1B* is not a major contributor to genetic susceptibility to RA.**

Interleukin-1 (IL-1) is a key mediator of inflammation, with pleiotropic effects on several cells and signaling pathways. The activity defined as IL-1 reflects the function of 2 molecules, IL-1α and IL-1β. *IL1A* encodes IL-1α, which is cell-bound, and *IL1B* encodes IL-1β, a secreted cytokine. IL-1 is a critical mediator in several systemic autoinflammatory syndromes and in juvenile rheumatoid arthritis, as evidenced by a dramatic effect of anti–IL-1 therapy in those diseases (1–3). IL-1

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also plays a pathogenic role in inflammation and tissue destruction in rheumatoid arthritis (RA) (4,5). IL-1 blockade ameliorates arthritis in multiple mouse models and has been shown in clinical trials to improve human RA (6).

Although the existence of genetic factors that modulate susceptibility to RA is well established, only a few such genes have thus far been identified and confirmed (7). Other than that for the "shared epitope" alleles at the HLA locus (8), the most compelling evidence of an association exists for *PTPN22* (9). Recently, a genome-wide association study not only confirmed the association between RA susceptibility and the *HLA* and *PTPN22* loci but also uncovered 9 other loci (at a significance level of $P = 10^{-5}$ to $P = 10^{-7}$) that are potentially associated with RA (10). In addition, single-nucleotide polymorphisms (SNPs) in the regions of *STAT4* (11,12), *TRAF1/C5* (13,14), and *TNFAIP3* (15,16) have recently been shown to be associated with RA susceptibility, but the functional consequence of these polymorphisms has not yet been elucidated.

The central role that IL-1 plays in inflammation suggests that allelic polymorphism in *IL1* might have an impact on susceptibility to RA. Indeed, in mice, allelic polymorphism in *Il1b* influences the severity of arthritis in the K/BxN model (17). In humans, the situation is less clear. A genome-wide scan of a family cohort showed an excess of allele sharing for the *IL1* gene cluster (18). A subsequent linkage study of the *IL1* locus showed no linkage with susceptibility to RA, although subgroup analysis demonstrated linkage of *IL1* in families with erosive RA if siblings did not share the *HLA–DRB1* allele (19). Similarly, case–control association studies revealed no association of *IL1* variants and susceptibility to RA but showed an association with severity or radiographic erosions or progression (19–24). The number of patients with RA who were screened in these studies was relatively small (<300 patients in all studies); therefore, these analyses were underpowered to show weak associations. In addition, these studies addressed a rather limited number of polymorphisms, focusing on some subset of only 2 polymorphisms in *IL1B* (−511 and +3954) and 2 polymorphisms in *IL1A* (−889 and +4845. These polymorphisms in *IL1A* are in tight linkage disequilibrium [LD] and thus provide the same information). International SNP databases currently list 88 SNPs in the *IL1B* gene alone; clearly, much of the existing variation has not yet been analyzed. In addition, it is possible that disease-specific variants found only in patients with RA exist and have not been catalogued in general SNP databases.

Given the importance of IL-1 as a mediator of inflammation, suggestive results from prior genetics studies in humans, and the clear evidence of an arthritis association in mouse models, we thought that a deeper and broader analysis of genetic variation at the *IL1* loci was warranted. Therefore, we performed a comprehensive and well-powered study to determine the relevance of polymorphisms in *IL1A* and *IL1B* to susceptibility to RA or to its destructive consequences.

PATIENTS AND METHODS

Clinical samples. Clinical samples were obtained from 5 sources, as follows:

1. Centre d'Etude du Polymorphisme Humain (CEPH) samples from the International HapMap project (90 individuals from Utah with ancestry from northern and western Europe) (25).
2. The Brigham Rheumatoid Arthritis Sequential Study (BRASS). A prospective longitudinal study of patients with RA diagnosed by a rheumatologist (26). Clinical measures, including anti–cyclic citrullinated peptide (anti-CCP) antibodies, rheumatoid factor (RF), C-reactive protein, and the Disease Activity Score in 28 joints (DAS28) (27), were obtained at the baseline visit. Hand radiographs were evaluated by a radiologist for the presence of erosions. Only the 774 patients who identified their race as "white" were selected for analysis, in order to avoid population admixture effects.
3. North American Rheumatoid Arthritis Consortium (NARAC). The cases included 1,314 individuals from 619 multiplex families (primarily affected sibling pairs); at least 1 sibling had documented erosions on hand radiographs, and at least 1 sibling experienced disease onset between the ages of 18 years and 60 years (28). All of the NARAC patients satisfied the 1987 American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria for the classification of RA (29). The control subjects were 1,103 individuals from the New York Cancer Project (NYCP), a population-based prospective study of healthy individuals. Approximately 2 control subjects were matched to a single randomly chosen affected sibling on the basis of sex, age (birth decade), and ethnicity (grandparental country/region of origin). (The number of cases does not equal the number of control subjects, because some case families contributed more or fewer than 2 siblings, and because 2 control subjects were not available for every case.) Only patients and control subjects who identified their ancestry as "white" were selected for this study. Complete data for *PTPN22* for this case–control sample set have been published previously (9).
4. The Spanish case–control sample set from the University Clinical Hospital of Santiago de Compostela. The study group comprised 525 patients satisfying the ACR criteria for the classification of RA (29) and 504 control subjects ages 55 years or older who were undergoing elective nonorthopedic surgery (30). The patients and control

subjects were white individuals of Spanish origin, all of whose known ancestors were of the same origin.

5. The Wichita Rheumatic Disease Data Bank (WRDDB)/National Inception Cohort of Rheumatoid Arthritis Patients (NICRAP)/Study of New Onset Rheumatoid Arthritis (SONORA) case–control sample set. Patients included 948 anti-CCP antibody–positive individuals in whom RA was diagnosed by a rheumatologist. These patients were selected from 3 independent registries, as follows: the WRDDB (in which the mean disease duration was 10 years) (31), the NICRAP (in which patients were enrolled within 6 months of the clinical diagnosis) (32), and SONORA (in which patients were enrolled within 3–12 months of the clinical diagnosis) (33). Control subjects included 1,455 unique individuals from the NYCP. Only patients and control subjects who identified their race as "white" were selected for this study.

Sequencing. The VISTA genome browser (34) was used to identify regions of homology with the rat and mouse genomes. Exons and noncoding regions showing homology with the rodent genomes were amplified by polymerase chain reaction (PCR) and then treated with exonuclease I and alkaline phosphatase. Bidirectional sequencing was performed. Sequencher software (Gene Codes Corp., Ann Arbor, MI) was used to align and compare chromatographs. Secondary peaks that were ≥30% of the maximum peak height were visually inspected for validity as heterozygotes.

Genotyping. Genotyping was performed primarily by primer extension of multiplex products, with detection by mass spectroscopy (35), using the Sequenom platform (36) and/or by allele-specific fluorogenic PCR (37). For quality control, *PTPN22* was genotyped by both methods in the BRASS registry (99.9% concordance). For fluorogenic PCR, the concordance of duplicates was 100%. All SNPs included in the analysis had a P value greater than 0.001 for Hardy-Weinberg distribution and a genotyping efficiency >70% (all but 4 of the SNPs genotyped in NARAC had a genotyping efficiency >95%). Individuals were excluded if >50% of the genotypes were missing. In NARAC, 28 individuals were excluded, with 96% of the remaining individuals genotyped at 80% of the markers and 74% genotyped at 90% of the markers. In the BRASS registry, 31 individuals were excluded, with 98% of the remaining individuals genotyped at 90% of the markers. Genotyping efficiency was 99.97% in the Spanish samples and 99.96% in the WRDDB/NICRAP/SONORA samples.

Statistical analysis. *Single-marker analysis.* P values were calculated for binary variables using 2×2 contingency tables of allele counts and chi-square testing. For continuous variables, P values were determined using linear regression (Plink; online at http://pngu.mgh.harvard.edu/~purcell/plink/ibdibs.shtml). Because >20 SNPs were tested in the NARAC and BRASS sample sets, we applied a conservative Bonferroni correction for multiple sampling, with a significance threshold of $P = 10^{-3}$.

LD analysis. LD was investigated using Haploview (38). Blocks of high LD were defined according to the method described by Gabriel et al (36).

Haplotype analysis. Phased haplotypes were reconstructed using 2 different statistical frameworks: expectation-maximization using Haploview (38) and Whap (39), and Bayesian using Phase (40,41). No significant differences were observed in the haplotypes derived using the different methods. For Whap, 500–1,000 repeats were performed for each analysis. Phase version 2.1.1 was run according to the authors' recommendations. For each set of SNPs tested as a haplotype, linear regression and a likelihood ratio test of overall association were performed to determine an omnibus P value. For omnibus P values ≤0.05, a haplotype-specific chi-square test was performed for each haplotype.

RESULTS

Sequencing of *IL1A* and *IL1B* in patients with RA. To understand the full genetic diversity within *IL1* in the context of RA, we sequenced *IL1A* and *IL1B* in a set of RA patients enrolled in the BRASS registry. Sequencing DNA from 24 patients (i.e., 48 chromosomes) provided 95% power to detect alleles with ≥5% frequency in the population (42). We performed bidirectional sequencing on all exons and promoter and noncoding regions that demonstrated a high degree of sequence similarity to the rat and mouse homologs (from the VISTA browser [34]) (Figure 1). The sequence data revealed 24 previously discovered SNPs, including 9 in *IL1B* and 15 in *IL1A*, and 4 novel SNPs in the noncoding regions of *IL1B* (submitted to the National Center for Biotechnology Information dbSNP database; accession nos. ss76859910, ss76859911, ss76859912, ss76859913). Hereafter, these SNPs will be referred to as RA1, RA2, RA3, and RA4, respectively. RA3 was subsequently reported as an SNP named "−5164" (43). RA1, RA2, RA3, and RA4 were identified in 1 of 48, 1 of 48, 10 of 48, and 2 of 48 sequenced chromosomes, respectively. All 4 novel SNPs were then verified by genotyping using allele-specific fluorogenic PCR in DNA obtained from the BRASS registry and other sample sets (see below). No novel SNPs were identified in *IL1A*.

Discovery case–control samples for analyzing association with RA susceptibility. Querying an association between *IL1* and RA by genotyping every known SNP in *IL1A* and *IL1B* would clearly be impractical. Instead, we followed a modified "tagging SNPs" strategy (44), which used a set of SNPs that captured the underlying diversity of the region based on the LD between SNPs, while also testing the novel and database-derived SNP alleles that had been observed by resequencing in patients with RA.

In order to comprehensively determine the LD structure in *IL1A* and *IL1B*, we genotyped a collection of SNPs in CEPH samples from the International HapMap project (25). This SNP set contained 15 of the 28

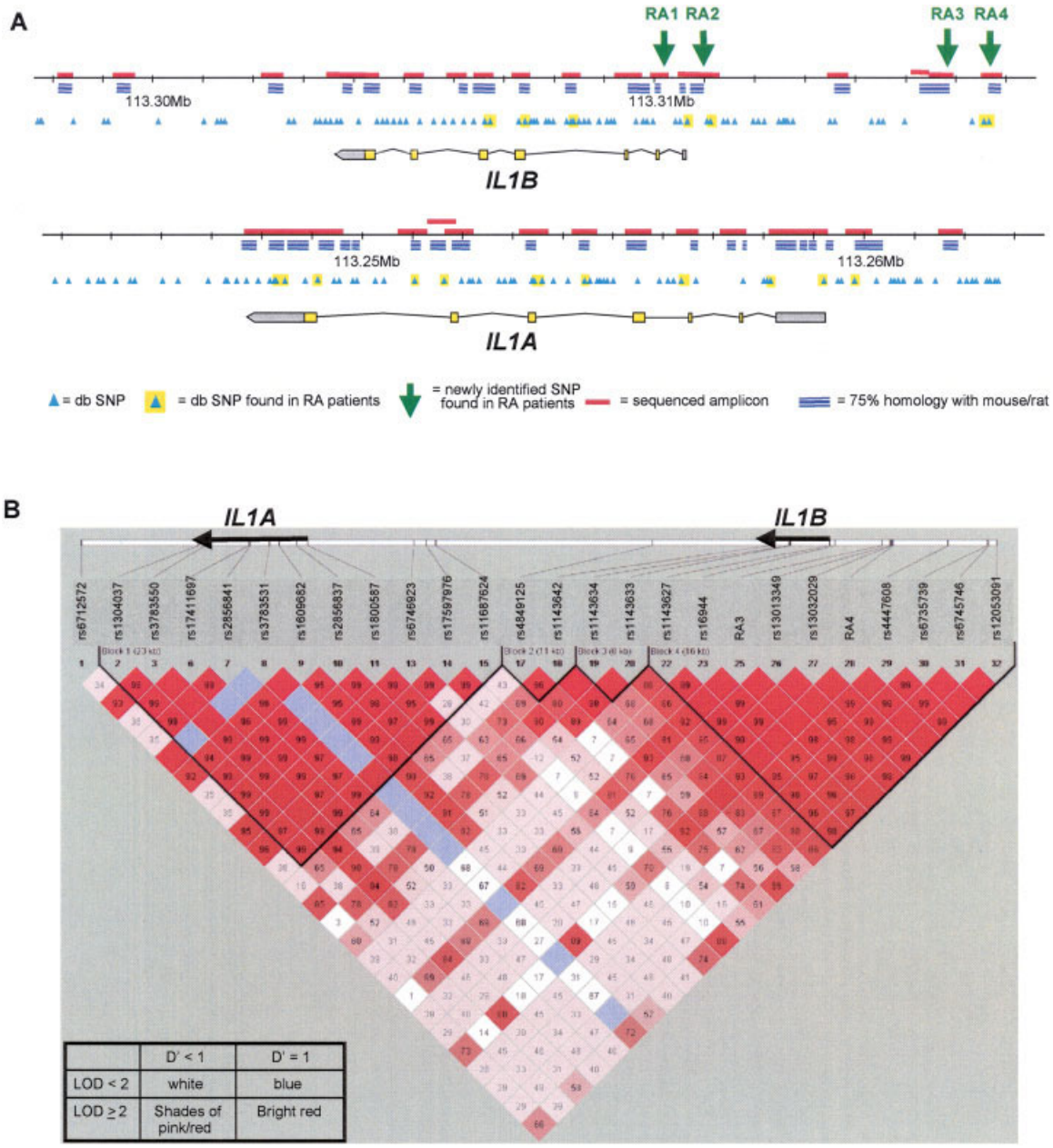

Figure 1. A, Schematic diagram of the *IL1A* and *IL1B* loci. **B,** Linkage disequilibrium map of the single-nucleotide polymorphisms (SNPs) genotyped in *IL1A* and *IL1B* in the North American Rheumatoid Arthritis Consortium, as displayed by the Haploview software package. Haplotype blocks (black lines) are drawn according to confidence intervals (35). D′ values are shown in the boxes. RA = rheumatoid arthritis; LOD = logarithm of odds.

SNPs identified by resequencing that were selected for multiplex genotyping reaction compatibility. Information for an additional 5 of the SNPs identified from *IL1A* and *IL1B* was already available in HapMap at that time. We also genotyped 23 additional database SNPs chosen from the 20-kb region around each gene to ensure complete coverage of the genetic diversity in these regions.

By collating the genotype data with information available from the HapMap project, we determined LD across the region (36,45). To select the final panel of SNPs to be genotyped in the case–control analysis, we

Table 1. Allelic polymorphism in *IL1* in NARAC cases and controls*

Gene/SNP	SNP characteristics	Alleles	Frequency (genotype counts)†		OR	95% CI	*P*‡
			Cases	Controls			
IL1A							
rs6712572	Intron (*CKAP2L*)	**T**/g	0.51 (332, 652, 296)	0.50 (282, 536, 276)	1.04	0.93–1.17	0.50
rs1304037	3′-UTR (*IL1A*)	**A**/g	0.73 (686, 506, 92)	0.70 (546, 444, 105)	1.16	1.02–1.32	0.021
rs3783550	Intron (*IL1A*)	**C**/a	0.31 (133, 530, 609)	0.30 (107, 416, 538)	1.08	0.95–1.22	0.26
rs17561	Missense (*IL1A*)	**G**/t	0.73 (686, 513, 87)	0.70 (545, 443, 104)	1.17	1.03–1.33	0.015
rs2856841	Intron (*IL1A*)	**T**/c	0.73 (673, 502, 91)	0.70 (537, 435, 107)	1.17	1.03–1.32	0.018
rs3783531	Missense (*IL1A*)	**A**/g	0.00 (0, 9, 1,282)	0.00 (0, 7, 1,089)	1.09	0.41–2.94	0.86
rs1609682	Intron (*IL1A*)	**C**/a	0.31 (142, 516, 621)	0.29 (110, 418, 559)	1.10	0.97–1.24	0.14
rs2856837	Intron (*IL1A*)	**C**/t	0.73 (686, 507, 90)	0.70 (560, 442, 104)	1.15	1.02–1.31	0.028
rs1800587	5′-UTR (*IL1A*)	**C**/t	0.73 (687, 507, 89)	0.70 (546, 445, 105)	1.17	1.03–1.33	0.015
rs6746923	5′-nongene region	**A**/g	0.42 (233, 615, 440)	0.40 (176, 535, 383)	1.06	0.94–1.19	0.34
rs17597976	5′-nongene region	**A**/g	0.13 (29, 283, 979)	0.12 (15, 242, 842)	1.08	0.91–1.28	0.40
rs11687624	5′-nongene region	**C**/t	0.44 (249, 643, 392)	0.42 (192, 532, 372)	1.10	0.98–1.24	0.090
IL1B							
rs4849125	3′-nongene region	**A**/g	0.70 (616, 539, 101)	0.69 (507, 444, 108)	1.08	0.95–1.23	0.23
rs1143642	Intron (*IL1B*)	**C**/t	0.93 (1,106, 171, 4)	0.92 (922, 163, 10)	1.22	0.98–1.51	0.075
rs1143634	Synonymous (*IL1B*)	**G**/a	0.78 (761, 416, 63)	0.78 (663, 378, 55)	1.02	0.89–1.17	0.76
rs1143633	Intron (*IL1B*)	**T**/c	0.37 (159, 580, 485)	0.37 (154, 493, 445)	1.00	0.89–1.13	0.94
RA1	Intron (*IL1B*)	**C**/t	1.00 (1,300, 0, 0)	1.00 (1,099, 0, 0)	NA	NA	NA
rs1143627	5′-nongene region	**A**/g	0.66 (544, 544, 149)	0.65 (465, 493, 135)	1.04	0.92–1.17	0.55
rs16944	5′-nongene region	**G**/a	0.66 (573, 548, 156)	0.65 (466, 501, 134)	1.06	0.94–1.20	0.35
RA2	5′-nongene region	**C**/t	1.00 (1,236, 0, 0)	1.00 (1,095, 1, 0)	NA	NA	0.29
RA3	5′-nongene region	**A**/g	0.84 (837, 302, 41)	0.80 (704, 341, 45)	1.27	1.09–1.48	0.0021
rs13013349	5′-nongene region	**G**/a	0.66 (537, 547, 153)	0.65 (467, 493, 135)	1.02	0.90–1.15	0.75
rs13032029	5′-nongene region	**T**/c	0.47 (198, 451, 250)	0.45 (190, 429, 276)	1.09	0.95–1.24	0.21
RA4	5′-nongene region	**C**/g	0.94 (829, 100, 2)	0.92 (778, 123, 6)	1.35	1.03–1.75	0.027
rs4447608	5′-nongene region	**T**/c	0.48 (203, 469, 250)	0.46 (200, 432, 270)	1.06	0.93–1.21	0.36
rs6735739	5′-nongene region	**C**/t	0.66 (568, 576, 156)	0.66 (475, 495, 130)	1.01	0.90–1.14	0.86
rs6745746	5′-nongene region	**A**/g	0.46 (228, 637, 332)	0.45 (222, 534, 329)	1.03	0.91–1.16	0.64
rs12053091	5′-nongene region	**C**/t	0.27 (77, 346, 499)	0.26 (69, 327, 504)	1.06	0.92–1.23	0.41

* Twenty-eight single-nucleotide polymorphisms (SNPs) from *IL1A* and *IL1B* were genotyped in 1,314 patients with rheumatoid arthritis from the North American Rheumatoid Arthritis Consortium (NARAC) and 1,103 control subjects. The alleles that are more frequent in cases are capitalized and shown in boldface, and the frequencies and odds ratios (ORs) are for those alleles. 95% CI = 95% confidence interval; UTR = untranslated region; NA = not applicable.

† The 3 numbers shown in parentheses are the genotype counts for homozygotes of the allele that is capitalized and shown in boldface, heterozygotes, and homozygotes of the alternate allele, respectively.

‡ *P* values are calculated from chi-square tests performed on allele counts for single markers in cases versus controls.

then used Tagger software (46) to choose SNPs that adequately represented all common haplotypes. We preferentially included SNPs of interest by constraining Tagger to choose the 4 novel SNPs in *IL1B* and the 24 database-derived SNPs that had been identified by sequencing in patients with RA, including 4 SNPs that had previously been associated with aggressive and/or erosive arthritis (*IL1B* −511 [rs16944], *IL1B* +3954 [rs1143634], *IL1A* −889 [rs1800587], and *IL1A* +4845 [rs17561]) (19–21,23,24). We then eliminated some SNPs that had perfect or near-perfect proxies in the set.

This process ultimately led to identification of a set of 28 SNPs, including a minimally redundant subset of those selected by constraining the tagging algorithm, as well as 11 additional SNPs chosen by Tagger to provide complete coverage of the major haplotypes. Based on the LD structure in the CEPH samples, this set captured 61 of 66 haplotypes in a 20-kb region surrounding each gene, with an r^2 value between the tagging SNPs and untested alleles of >0.8 and a mean r^2 value of 0.984.

The 28 SNPs were genotyped in 1,314 RA patients from NARAC, which was used as our "discovery" sample set, because its RA patients have severe disease, with ~95% having evidence of joint erosions on hand radiographs. A total of 1,103 control samples were selected from the NYCP, as described previously (9). In a dominant model with 10% allele frequency, we had 90% power to detect a relative risk of 1.5 (47). The data were analyzed (by chi-square test) for correlation between the individual SNPs and susceptibility to RA (Table 1). Five SNPs in *IL1A* and 2 SNPs in *IL1B* showed differences between patients and control subjects, although none reached a Bonferroni-corrected

Table 2. Allelic polymorphism in RA3 and RA4 in replication-set cases and controls*

SNP	Allele	Frequency (genotype counts)†		OR	95% CI	*P*‡
		Cases	Controls			
WRDDB/NICRAP/SONORA						
RA3	**A**/g	0.79 (589, 322, 37)	0.80 (940, 452, 62)	0.94	0.81–1.08	0.39
RA4	**C**/g	0.94 (844, 104, 0)	0.93 (1,261, 194, 0)	1.23	0.96–1.57	0.097
Spanish cohort						
RA3	**A**/g	0.77 (310, 185, 26)	0.79 (318, 166, 20)	0.88	0.71–1.08	0.22
RA4	**C**/g	0.92 (432, 73, 4)	0.91 (415, 83, 5)	1.18	0.86–1.61	0.30

* RA3 (ss76859912) and RA4 (ss76859913) were genotyped in 2 independent replication case–control sample sets. First, they were genotyped in 948 anti–cyclic citrullinated peptide antibody–positive patients from the Wichita Rheumatic Disease Data Bank (WRDDB)/National Inception Cohort of Rheumatoid Arthritis Patients (NICRAP)/Study of New Onset Rheumatoid Arthritis (SONORA) cohort and 1,455 control subjects from the New York Cancer Project. Next, they were genotyped in 525 patients with rheumatoid arthritis and 504 control subjects from northern Spain. Frequencies and odds ratios (ORs) are for the alleles that are capitalized and shown in boldface. 95% CI = 95% confidence interval.
† The 3 numbers shown in parentheses are the genotype counts for homozygotes of the allele that is capitalized and shown in boldface, heterozygotes, and homozygotes of the alternate allele, respectively.
‡ *P* values are from chi-square tests performed on allele counts for single markers in cases versus controls.

threshold for significance ($P = 0.001$). The 5 SNPs in *IL1A* are in significant LD, with r^2 values of 0.98–0.99 in the NARAC case–control data set. The most significant association was observed for RA3, with an odds ratio (OR) of 1.27 (95% confidence interval [95% CI] 1.09–1.48 [$P = 0.0021$]). Of the other SNPs initially identified by sequencing of RA samples, RA1 was shown to be monomorphic in all patients and control subjects, and the rare RA2 variant was observed in only 1 control subject. Because the NARAC collection includes affected siblings, it is possible that the OR could be inflated by using a simple case–control model. To examine this possibility, we performed the same analysis using only 1 sibling from each family (n = 619). The ORs for RA3 and RA4 were 1.37 (95% CI 1.13–1.67 [$P = 0.0015$]) and 1.49 (95% CI 1.05–2.11 [$P = 0.0242$]), respectively, suggesting that inclusion of siblings did not exaggerate the OR.

Recent studies have demonstrated that the high-risk alleles at *HLA* and *PTPN22* are more significantly associated with susceptibility to RA in the subset of patients who are positive for antibodies against CCP (48). We therefore investigated the degree of association of *IL1* polymorphisms with RA in subgroups of patients defined by anti-CCP antibody status. No increased association was observed when we examined anti-CCP antibody–positive patients versus control subjects or RF-positive patients versus control subjects, or after stratification for *HLA* susceptibility alleles (HLA–DRB1*0101, *0401, *0404, *0405, *0408, or *1001) or sex (data not shown). However, when the patients and control subjects were stratified according to their *PTPN22* genotype, the association signal for RA3 increased in the subset (912 patients and 933 control subjects) that was homozygous for the low-risk *PTPN22* allele (OR 1.43, 95% CI 1.19–1.71 [$P = 0.0000897$], with allele frequencies for RA3/A of 0.85 in patients and 0.80 in control subjects). Conversely, the OR for RA4 and, to a lesser extent, rs1143642, was enhanced in the 382 patients and 158 control subjects with at least 1 copy of the high-risk allele at *PTPN22* (for RA4, OR 2.48, 95% CI 1.37–4.48 [$P = 0.002$], with allele frequencies for RA4/C of 0.959 in patients and 0.905 in control subjects; for rs1143642, OR 1.68, 95% CI 1.03–2.72 [$P = 0.0344$], with allele frequencies of 0.941 in patients and 0.905 in control subjects).

Case–control replication of association with RA susceptibility. We attempted to replicate the association in 2 additional independent case–control sample sets by testing RA3 (which has the strongest suggestive association with susceptibility to RA) and RA4 (a novel and possibly RA-specific SNP variant). The first replication set included 948 anti-CCP antibody–positive individuals combined from 3 independent registries (WRDDB/NICRAP/SONORA) (31–33) and 1,455 unique control subjects from the NYCP. The second replication set comprised 525 patients and 504 control subjects from the University Clinical Hospital of Santiago de Compostela, Spain (30).

There was no population-specific variation in allele frequencies for RA3 and RA4 when comparing the NARAC and the replication samples. However, in contrast to the findings in NARAC, the frequency of the RA3/A disease-associated allele in the replication samples was actually lower in the patients with RA compared with control subjects, particularly in the Spanish patients (Table 2). For RA4, the higher frequency of the C allele in patients with RA was reproduced in the 2

Table 3. Allelic polymorphism in *IL1* in BRASS patients with and those without erosions*

Gene/SNP	SNP characteristics	Alleles	Frequency (genotype counts)†		OR	95% CI	*P*‡
			Erosion positive	Erosion negative			
IL1A							
rs6716572	Intron (*CKAP2L*)	**T**/g	0.52 (108, 209, 93)	0.51 (72, 154, 71)	1.06	0.86–1.32	0.58
rs3783550	Intron (*IL1A*)	**C**/a	0.28 (34, 160, 203)	0.28 (24, 115, 153)	1.01	0.79–1.25	0.96
rs17561	Missense (*IL1A*)	**G**/t	0.74 (223, 157, 30)	0.70 (145, 117, 34)	1.23	0.97–1.60	0.09
rs3783531	Missense (*IL1A*)	**A**/g	0.00 (0, 3, 406)	0.00 (0, 3, 293)	0.70	0.10–2.83	0.72
rs1894399	Intron (*IL1A*)	**G**/a	0.74 (220, 155, 26)	0.71 (145, 116, 29)	1.18	0.93–1.55	0.18
rs1800587	5′-UTR (*IL1A*)	**C**/t	0.74 (222, 158, 30)	0.70 (146, 118, 34)	1.21	0.95–1.57	0.12
rs6746923	5′-nongene region	**A**/g	0.45 (80, 200, 127)	0.40 (50, 136, 110)	1.20	0.96–1.49	0.10
rs17597976	5′-nongene region	**A**/g	0.14 (4, 104, 294)	0.13 (7, 61, 226)	1.12	0.81–1.46	0.49
IL1B							
rs4849125	3′-nongene region	**A**/g	0.72 (213, 155, 37)	0.68 (137, 119, 37)	1.22	0.96–1.57	0.10
rs7596684	3′-nongene region	**T**/c	0.82 (271, 117, 17)	0.79 (180, 97, 16)	1.19	0.91–1.61	0.21
rs1143634	Synonymous (*IL1B*)	**G**/a	0.79 (252, 129, 19)	0.78 (167, 106, 10)	1.09	0.84–1.45	0.54
rs1143633	Intron (*IL1B*)	**T**/c	0.38 (58, 191, 150)	0.36 (34, 135, 114)	1.12	0.89–1.38	0.33
RA1	Intron (*IL1B*)	**C**/t	1.00 (399, 0, 0)	1.00 (281, 1, 0)	NA	NA	0.23
rs1143627	5′-nongene region	**A**/g	0.67 (181, 168, 44)	0.66 (120, 129, 31)	1.07	0.85–1.37	0.56
rs16944	5′-nongene region	**G**/a	0.67 (177, 177, 44)	0.66 (118, 134, 30)	1.05	0.84–1.34	0.67
RA3	5′-nongene region	**A**/g	0.80 (254, 125, 17)	0.82 (189, 84, 8)	0.86	0.65–1.17	0.29
rs13013349	5′-nongene region	**G**/a	0.67 (180, 176, 44)	0.66 (119, 136, 28)	1.04	0.83–1.33	0.72
rs13032029	5′-nongene region	**T**/c	0.48 (93, 196, 107)	0.46 (56, 142, 80)	1.11	0.89–1.37	0.36
RA4	5′-nongene region	**C**/g	0.94 (357, 36, 5)	0.91 (232, 49, 0)	1.56	1.03–2.58	0.036
rs4447608	5′-nongene region	**T**/c	0.49 (94, 198, 106)	0.46 (55, 149, 76)	1.09	0.88–1.36	0.42
rs6735739	5′-nongene region	**C**/t	0.67 (182, 172, 44)	0.66 (118, 138, 26)	1.05	0.83–1.34	0.69
rs6745746	5′-nongene region	**A**/g	0.47 (82, 208, 105)	0.45 (46, 160, 76)	1.10	0.89–1.37	0.38
rs12053091	5′-nongene region	**C**/t	0.25 (23, 148, 215)	0.23 (17, 89, 161)	1.12	0.87–1.41	0.39

* Twenty-four SNPs from *IL1A* and *IL1B* were genotyped in 712 patients with rheumatoid arthritis from the Brigham Rheumatoid Arthritis Sequential Study (BRASS). A chi-square analysis was performed comparing *IL1* allele counts in the 403 patients with erosions seen on plain radiographs of the hands and the 309 patients who did not have erosions. Frequencies and ORs are for the alleles that are capitalized and shown in boldface. See Table 1 for other definitions.

† The 3 numbers shown in parentheses are the genotype counts for homozygotes of the allele that is capitalized and shown in boldface, heterozygotes, and homozygotes of the alternate allele, respectively.

‡ *P* values are from chi-square tests performed on allele counts for single markers in cases versus controls.

replication sample sets, but these differences did not reach statistical significance ($P = 0.09$ and $P = 0.3$), regardless of *PTPN22* stratification (data not shown).

***IL1* polymorphism and disease characteristics.** Our second question was whether certain alleles of *IL1* are associated with more severe or erosive disease, as suggested by prior studies involving smaller numbers of patients (19–24). We examined the association between the SNP alleles at *IL1A* and *IL1B* and the presence of erosions on hand radiographs in patients enrolled in the BRASS registry. For genotyping, we again chose a set of "tag" SNPs in *IL1A* and *IL1B*, based on LD structure in CEPH, with priority given to SNPs discovered by sequencing in RA samples. (The procedure used to identify SNPs was the same as that used for the NARAC set, but, for technical reasons, the panel of SNPs was slightly different.) We again included the 4 SNPs previously associated with aggressive or erosive arthritis (19–21,23,24). When analyzed in the CEPH samples, the 23 genotyped SNPs together captured 61 of 66 haplotypes in a 20-kb region surrounding each gene, with an r^2 value between the tagging and untested alleles of >0.8 and a mean r^2 value of 0.987.

In the BRASS cohort, 403 patients were found to have erosions, whereas 309 patients did not have erosions (80% power to detect a relative risk of 1.6). There was no association between erosion status and the previously reported *IL1B* −511, *IL1B* +3954, *IL1A* +4845, and *IL1A* −889 variants. The strongest association, which did not meet a corrected significance threshold of $P = 0.001$, was observed for the C allele at RA4 (OR 1.56, 95% CI 1.03–2.58 [$P = 0.036$]) (Table 3). In order to confirm this finding, we compared cases of erosive disease (n = 142) and cases of nonerosive disease (n = 272) in the cohort of Spanish patients for whom data on erosion status were available. In that cohort, RA4/C showed a frequency of 0.927 in the patients with erosive disease and a frequency of 0.942 in patients with nonerosive disease, for an OR of 0.78; these findings are in the opposite direction from those in the BRASS cohort.

Further analysis in the BRASS cohort revealed

Table 4. *IL1A* haplotype association with NARAC cases and controls*

Haplotype	No. of cases	No. of controls	Frequency		OR	95% CI	*P*
			Cases	Controls			
IL1A							
GACGTGCCCGGC	776	607	0.30	0.28	1.12	0.99–1.27	0.083
TAAGTGACCAGT	722	596	0.28	0.27	1.04	0.91–1.18	0.59
GGATCGATTGGT	443	443	0.17	0.20	0.82	0.70–0.94	0.006
TAAGTGACCAAC	319	261	0.12	0.12	1.04	0.87–1.24	0.66
TGATCGATTGGT	229	203	0.09	0.09	0.95	0.78–1.16	0.62
TACGTGCCCGGC	14	26	0.01	0.01	0.45	0.24–0.87	0.015
GAAGTGACCAGT	10	20	0.00	0.01	0.42	0.20–0.90	0.022
Remaining haplotypes	83	42	0.03	0.02	–	–	–

* All of the single-nucleotide polymorphisms (SNPs) within the 20-kb region of *IL1A* were used to construct haplotypes for the North American Rheumatoid Arthritis Consortium (NARAC) cases and controls. Haplotypes with a frequency of ≥0.005 are shown. SNPs are ordered as shown in Table 1. OR = odds ratio; 95% CI = 95% confidence interval.

no significant association when anti-CCP antibody–positive versus anti-CCP antibody–negative cases were compared, nor a correlation of any of the *IL1* SNPs with anti-CCP antibody levels, RF levels, or the DAS28 (data not shown).

Haplotype analysis. Because the analysis described above showed no reproducible association between polymorphism at single markers and the susceptibility to or severity of RA, we reconstructed phased haplotypes in the study subjects and searched for association between these haplotypes and disease incidence or characteristics.

For NARAC patients and control subjects, we determined haplotypes defined by all of the SNPs, the SNPs around *IL1A*, and the SNPs around *IL1B*. For the extended *IL1A* haplotype, regression analysis provided an omnibus *P* value of 0.007, with 1 haplotype (GGATCGATTGGT) occurring in 17% of patients and 20% of control subjects (OR 0.82, 95% CI 0.70–0.94 [*P* = 0.006]) (Table 4). If only a single sibling from each family of cases was included, the magnitude of the OR for that haplotype was unchanged (OR 0.81, 95% CI 0.68–0.98 [*P* = 0.026]), suggesting that the potential association was not attributable to merely the inclusion of related individuals. In contrast to *IL1A*, the regression analysis for the extended *IL1B* haplotype and the extended *IL1A–IL1B* haplotype revealed *P* values of 0.295 and 0.233, respectively, suggesting no statistically significant skewing in the haplotype distribution. The haplotype-specific results (by chi-square test) for the extended *IL1A–IL1B* haplotype are shown in Table 5.

In addition, we analyzed SNP haplotypes defined

Table 5. Extended *IL1A–IL1B* haplotype association with NARAC cases and controls*

Haplotype	No. of cases	No. of controls	Frequency		OR	95% CI	*P*
			Cases	Controls			
IL1A–IL1B							
TAAGTGACCAGT_ACGTCAGCAGTCTCAT	576	476	0.22	0.22	1.02	0.89–1.17	0.76
GACGTGCCCGGC_ACGCCGACAACCCTGC	456	361	0.17	0.16	1.07	0.92–1.25	0.35
GACGTGCCCGGC_ACGCCAGCAGTCTCAT	229	149	0.09	0.07	1.32	1.06–1.63	0.011
GGATCGATTGGT_GCACCAGCGGCCCGGT	198	186	0.08	0.08	0.89	0.72–1.09	0.25
TGATCGATTGGT_GCACCAGCGGCCCCGT	155	141	0.06	0.06	0.92	0.73–1.16	0.48
TAAGTGACCAAC_ACGTCAGCAGTCTCAT	139	97	0.05	0.04	1.22	0.93–1.59	0.15
TAAGTGACCAAC_GTGCCGACAACCCTGT	110	105	0.04	0.05	0.87	0.67–1.15	0.34
GGATCGATTGGT_ACGTCAGCAGTCTCAT	84	92	0.03	0.04	0.76	0.56–1.03	0.073
GGATCGATTGGT_GCACCGACAACCCTGC	51	36	0.02	0.02	1.19	0.78–1.84	0.42
TAAGTGACCAGT_ACACCAGCGGCCCCGT	42	30	0.02	0.01	1.18	0.74–1.89	0.49
GGATCGATTGGT_ACGCCGACAACCCTGC	41	52	0.02	0.02	0.66	0.43–0.99	0.045
GACGTGCCCGGC_ACGTCAGCAGTCTCAT	40	43	0.02	0.02	0.78	0.50–1.20	0.26
TAAGTGACCAAC_GTGCCAGCAGTCTCAT	24	27	0.01	0.01	0.74	0.43–1.29	0.29
Remaining haplotypes	483	413	0.18	0.19			

* All of the SNPs genotyped from *IL1A* and *IL1B* were used to construct haplotypes for the NARAC cases and controls. Haplotypes with a frequency of ≥0.01 are shown. SNPs are ordered as shown in Table 1. See Table 4 for definitions.

as blocks determined by D′ confidence intervals (35) (Figure 1B), none of which provided an omnibus *P* value <0.05 in regression analysis (data not shown).

We similarly tested phased haplotypes reconstructed in the BRASS cohort for an association with erosion and other clinical variables. The omnibus *P* value for the regression was >0.05 for association with erosion status, anti-CCP antibodies, RF, and the DAS28 (data not shown).

DISCUSSION

Previous studies suggested an association between polymorphisms at *IL1* and severe, erosive RA. We sought to perform a comprehensive, more definitive study adequately powered to determine whether *IL1A* or *IL1B* contributes to the risk or severity of RA. *IL1A* and *IL1B* were sequenced in patients with RA to fully assess the potential disease-relevant genetic diversity, and tagging SNPs that captured the majority of this diversity were chosen for further assessment. In the single-marker analysis, involving more than 3,500 patients and 3,000 control subjects in 4 independent sample sets, we observed no association between *IL1* and RA susceptibility/severity that proved reproducible and met rigorous criteria for significance after correction for multiple comparisons.

The SNP demonstrating the strongest association with susceptibility to RA in the primary NARAC case–control set was RA3. Unfortunately, this association of RA3/A with susceptibility to RA was not validated in the replication samples. In fact, the allele distribution between patients and control subjects was in the opposite direction in the other 2 populations, suggesting that the lack of replication was not attributable to insufficient power only. The most likely explanation for the discrepancy is that the initial finding was a false positive and not representative of a true association. One alternative explanation is that the patients in the replication sets differed significantly from the discovery patients, and the association with disease susceptibility is only relevant in a subset of patients that was enriched in the NARAC cohort. Indeed, the patients in NARAC do represent a subset of RA patients with severe and aggressive disease, and, therefore, it is possible that the association with RA3 may be relevant only for severe erosive RA. If this were true, we would have expected to observe in the BRASS cohort an association of RA3/A with erosion status, which was not the case. If anything, the alternate RA3/G allele was associated with erosion. Theoretically, one could invoke the possibility that ethnic diversity is contributing to the difference between NARAC and the replication sample sets, but the allele frequencies for RA3 were similar in all groups.

Our analysis of the NARAC case–control samples was designed to have 90% power to detect a relative risk of 1.5 in a dominant model of an allele with 10% frequency (47). Therefore, our study was somewhat underpowered to detect weak associations with RA4 and other rare alleles. In fact, RA4/C showed a 1–2% greater frequency in patients than in control subjects in all 3 sample sets, and this difference was enhanced to 2–5% when stratified for the high-risk *PTPN22* allele (Tables 1 and 2, and data not shown). Based on the *P* values (by chi-square test) and a conservative Bonferroni correction for the number of comparisons, this difference is not statistically significant.

Interestingly, the same allele was potentially associated with erosive RA versus nonerosive RA in the BRASS cohort, with RA4/C being 3% more frequent in patients with erosive disease (uncorrected $P = 0.03$). This potential association with erosion, however, was not replicated in the Spanish cohort. One possible issue concerning the use of erosion status as a marker of disease severity is that a radiograph is obtained at one point in time, and that erosive disease may ultimately develop in some of the patients who are erosion-negative or in those patients who would have had erosions in the absence of aggressive therapy. Therefore, the readout for each patient is influenced by the length of therapy, the severity of disease, and the aggressiveness of treatment.

Previous studies had shown association of rs16944, rs1143634, rs1800587, and rs17561 with erosive arthritis or radiographic progression, but the associated SNP and sometimes the associated allele differed among those studies (19–21,23,24). The disparities between these prior studies may suggest that the original findings were false-positive results reflective of the small number of patients studied. Indeed, our study in the BRASS cohort showed no statistically significant evidence of association with erosive disease status for any of these SNPs.

Although the single-marker analysis did not reveal a significant association between RA and *IL1A*, the haplotype analysis did uncover an *IL1A* haplotype, which was present in 17% of patients and 20% of control subjects, with a potential association with disease susceptibility. This finding could suggest that a certain set of SNP alleles may alter IL-1 function in an additive or synergistic manner or may indicate that a relevant allele is incompletely tagged with any of our single markers but

is represented by one of the complex haplotypes. Because we did not genotype the entire set of *IL1A* and *IL1B* SNPs in the replication sample sets, we could not validate the haplotype association. At this point, we cannot exclude the possibility that certain haplotypes at *IL1A* may contribute to RA susceptibility. If this is the case, however, the magnitude of the effect is likely to be small.

We did not examine the relevance of polymorphisms in *IL1RN*, the gene that encodes the competitive antagonist of IL-1α and IL-1β, IL-1 receptor antagonist (IL-1Ra). In fact, similar to the case with *IL1A* and *IL1B*, small studies have suggested an association with polymorphism in *IL1RN* and RA susceptibility and/or severity (49,50), but results have been contradictory (20,21), and therefore replication is needed. Given the importance of IL-1 in inflammatory arthritis, it may be interesting to perform a well-powered candidate gene study to examine the contribution of polymorphisms in other members of the IL-1 pathway, including the genes for the IL-1 receptors, IL-Ra, and components of the inflammasome.

In summary, based on our comprehensive study of variation at the *IL1A* and *IL1B* loci and genotyping in a large number of patients with RA and control subjects, we observed no evidence that common variants in *IL1* contribute significantly to the risk or severity of RA in the majority of patients.

AUTHOR CONTRIBUTIONS

Dr. Mathis had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Johnsen, Shadick, Weinblatt, Benoist, Mathis.

Acquisition of data. Johnsen, Plenge, Campbell, Dieguez-Gonzalez, Gomez-Reino, Shadick, Weinblatt, Gonzalez, Gregersen.

Analysis and interpretation of data. Johnsen, Plenge, Butty, Dieguez-Gonzalez, Gomez-Reino, Weinblatt, Gonzalez, Benoist, Mathis.

Manuscript preparation. Johnsen, Plenge, Butty, Weinblatt, Benoist, Mathis.

Statistical analysis. Johnsen, Plenge, Butty.

Provision of samples. Shadick.

REFERENCES

1. Dinarello CA. The many worlds of reducing interleukin-1 [editorial]. Arthritis Rheum 2005;52:1960–7.
2. Dinarello CA. Blocking IL-1 in systemic inflammation [review]. J Exp Med 2005;201:1355–9.
3. Fitzgerald AA, Leclercq SA, Yan A, Homik JE, Dinarello CA. Rapid responses to anakinra in patients with refractory adult-onset Still's disease. Arthritis Rheum 2005;52:1794–803.
4. Goldring SR. Pathogenesis of bone and cartilage destruction in rheumatoid arthritis. Rheumatology (Oxford) 2003;42 Suppl 2:ii11–6.
5. Dayer JM. The pivotal role of interleukin-1 in the clinical manifestations of rheumatoid arthritis. Rheumatology (Oxford) 2003;42 Suppl 2:ii3–10.
6. Bresnihan B, Cobby M. Clinical and radiological effects of anakinra in patients with rheumatoid arthritis. Rheumatology (Oxford) 2003;42 Suppl 2:ii22–8.
7. Gregersen PK. Teasing apart the complex genetics of human autoimmunity: lessons from rheumatoid arthritis [review]. Clin Immunol 2003;107:1–9.
8. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30:1205–13.
9. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 2004;75:330–7.
10. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–78.
11. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. N Engl J Med 2007;357:977–86.
12. Lee HS, Remmers EF, Le JM, Kastner DL, Bae SC, Gregersen PK. Association of STAT4 with rheumatoid arthritis in the Korean population. Mol Med 2007;13:455–60.
13. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis: a genomewide study. N Engl J Med 2007;357:1199–209.
14. Kurreeman FA, Padyukov L, Marques RB, Schrodi SJ, Seddighzadeh M, Stoeken-Rijsbergen G, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis [published erratum appears in PLoS Med 2007;4:e358]. PLoS Med 2007;4:e278.
15. Plenge RM, Cotsapas C, Davies L, Price AL, de Bakker PI, Maller J, et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. Nat Genet 2007;39:1477–82.
16. Thomson W, Barton A, Ke X, Eyre S, Hinks A, Bowes J, et al. Rheumatoid arthritis association at 6q23. Nat Genet 2007;39:1431–3.
17. Ohmura K, Johnsen A, Ortiz-Lopez A, Desany P, Roy M, Besse W, et al. Variation in IL-1β gene expression is a major determinant of genetic differences in arthritis aggressivity in mice. Proc Natl Acad Sci U S A 2005;102:12489–94.
18. Barrera P, Faure S, Prud'homme JF, Balsa A, Migliorini P, Chimenti D, et al, for the European Consortium on Rheumatoid Arthritis Families (ECRAF). European genetic study on rheumatoid arthritis: is there a linkage of the interleukin-1 (IL-1), IL-10 or IL-4 genes to RA? Clin Exp Rheumatol 2001;19:709–14.
19. Cox A, Camp NJ, Cannings C, Di Giovine FS, Dale M, Worthington J, et al. Combined sib-TDT and TDT provide evidence for linkage of the interleukin-1 gene cluster to erosive rheumatoid arthritis. Hum Mol Genet 1999;8:1707–13.
20. Buchs N, Di Giovine FS, Silvestri T, Vannier E, Duff GW, Miossec P. IL-1B and IL-1Ra gene polymorphisms and disease severity in rheumatoid arthritis: interaction with their plasma levels. Genes Immun 2001;2:222–8.
21. Cantagrel A, Navaux F, Loubet-Lescoulie P, Nourhashemi F, Enault G, Abbal M, et al. Interleukin-1β, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: relationship to occurrence and severity of rheumatoid arthritis. Arthritis Rheum 1999;42:1093–100.
22. Cvetkovic JT, Wallberg-Jonsson S, Stegmayr B, Rantapaa-Dahlqvist S, Lefvert AK. Susceptibility for and clinical manifestations of rheumatoid arthritis are associated with polymorphisms of the TNF-α, IL-1β, and IL-1Ra genes. J Rheumatol 2002;29:212–9.
23. Genevay S, Di Giovine FS, Perneger TV, Silvestri T, Stingelin S,

Duff G, et al. Association of interleukin-4 and interleukin-1B gene variants with Larsen score progression in rheumatoid arthritis. Arthritis Rheum 2002;47:303–9.
24. Jouvenne P, Chaudhary A, Buchs N, Giovine FS, Duff GW, Miossec P. Possible genetic association between interleukin-1α gene polymorphism and the severity of chronic polyarthritis. Eur Cytokine Netw 1999;10:33–6.
25. The International HapMap Consortium. The International HapMap Project. Nature 2003;426:789–96.
26. Shadick NA, Heller JE, Weinblatt ME, Maher NE, Cui J, Ginsburg GS, et al. Opposing effects of the D70 mutation and the shared epitope in HLA-DR4 on disease activity and certain disease phenotypes in rheumatoid arthritis. Ann Rheum Dis 2007;66:1497–502.
27. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight–joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8.
28. Jawaheer D, Seldin MF, Amos CI, Chen WV, Shigeta R, Etzel C, et al, for the North American Rheumatoid Arthritis Consortium. Screening the genome for rheumatoid arthritis susceptibility genes: a replication study and combined analysis of 512 multicase families. Arthritis Rheum 2003;48:906–16.
29. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
30. Rodriguez-Lopez J, Perez-Pampin E, Gomez-Reino JJ, Gonzalez A. Regulatory polymorphisms in extracellular matrix protease genes and susceptibility to rheumatoid arthritis: a case-control study. Arthritis Res Ther 2006;8:R1.
31. Wolfe F, Michaud K, Gefeller O, Choi HK. Predicting mortality in patients with rheumatoid arthritis. Arthritis Rheum 2003;48:1530–42.
32. Fries JF, Wolfe F, Apple R, Erlich H, Bugawan T, Holmes T, et al. HLA–DRB1 genotype associations in 793 white patients from a rheumatoid arthritis inception cohort: frequency, severity, and treatment bias. Arthritis Rheum 2002;46:2320–9.
33. Bombardier C, Deaton RL, Gregerson P, Massarotti E, Formica C, Weisman MH. Pattern of DMARD use in a North American cohort of patients with early rheumatoid arthritis (RA) (SONORA) [abstract]. Arthritis Rheum 2002;46 Suppl 9:S344.
34. Frazer KA, Pachter L, Poliakov A, Rubin EM, Dubchak I. VISTA: computational tools for comparative genomics. Nucleic Acids Res 2004;32(Web Server issue):W273–9.
35. Tang K, Fu DJ, Julien D, Braun A, Cantor CR, Koster H. Chip-based genotyping by mass spectrometry. Proc Natl Acad Sci U S A 1999;96:10016–20.
36. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. Science 2002;296:2225–9.
37. Zucchelli S, Holler P, Yamagata T, Roy M, Benoist C, Mathis D. Defective central tolerance induction in NOD mice: genomics and genetics. Immunity 2005;22:385–96.
38. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–5.
39. Purcell S, Daly MJ, Sham PC. WHAP: haplotype-based association analysis. Bioinformatics 2007;23:255–6.
40. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89.
41. Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;73:1162–9.
42. Kruglyak L, Nickerson DA. Variation is the spice of life. Nat Genet 2001;27:234–6.
43. Chen H, Wilkins LM, Aziz N, Cannings C, Wyllie DH, Bingle C, et al. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. Hum Mol Genet 2006;15:519–29.
44. De Bakker PI, Burtt NP, Graham RR, Guiducci C, Yelensky R, Drake JA, et al. Transferability of tag SNPs in genetic association studies in multiple populations. Nat Genet 2006;38:1298–303.
45. Daly MJ, Rioux JD, Schaffner SF, Hudson TJ, Lander ES. High-resolution haplotype structure in the human genome. Nat Genet 2001;29:229–32.
46. De Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet 2005;37:1217–23.
47. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003;19:149–50.
48. Plenge RM, Padyukov L, Remmers EF, Purcell S, Lee AT, Karlson EW, et al. Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. Am J Hum Genet 2005;77:1044–60.
49. Carreira PE, Gonzalez-Crespo MR, Ciruelo E, Pablos JL, Santiago B, Gomez-Camara A, et al. Polymorphism of the interleukin-1 receptor antagonist gene: a factor in susceptibility to rheumatoid arthritis in a Spanish population. Arthritis Rheum 2005;52:3015–9.
50. Kapetanovic MC, Lindqvist E, Sturfelt G, Saxne T, Truedsson L, Eberhardt K. The impact of IL-1Ra and MBL gene polymorphisms on joint damage after 5 and 10 years in patients with early rheumatoid arthritis. J Rheumatol 2007;34:894–5.