Am. J. Hum. Genet. 75:587–595, 2004

The Interleukin 1 Gene Cluster Contains a Major Susceptibility Locus for Ankylosing Spondylitis

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Ankylosing spondylitis (AS) is a common and highly heritable inflammatory arthropathy. Although the gene *HLA-B27* is almost essential for the inheritance of the condition, it alone is not sufficient to explain the pattern of familial recurrence of the disease. We have previously demonstrated suggestive linkage of AS to chromosome 2q13, a region containing the interleukin 1 (IL-1) family gene cluster, which includes several strong candidates for involvement in the disease. In the current study, we describe strong association and transmission of IL-1 family gene cluster single-nucleotide polymorphisms and haplotypes with AS.

Introduction

Ankylosing spondylitis (AS [MIM 106300]) affects 1–9 in 1,000 British white individuals, making it one of the most common causes of inflammatory arthritis after rheumatoid arthritis (van der Linden et al. 1983; Braun et al. 1998). The condition affects predominantly the axial skeleton, including the spine and sacroiliac joints, and causes pain, stiffness, and, eventually, bony ankylosis. Joints and tendon insertions (entheses) elsewhere are also commonly involved, and approximately one-third of patients develop acute anterior uveitis.

Familial clustering has implicated genetic factors in the disease etiology; heritability, as assessed by twin studies, is >90% (Brown et al. 1997), and the sibling recurrence risk ratio is 53–82 (Brown et al. 2000*b*). The major susceptibility gene in AS is *HLA-B27* (Brewerton et al. 1973). *HLA-B27* carriage is present in >95% of white individuals with AS, yet only 1%–5% of *HLA-B27* carriers develop AS, and *HLA-B27* carriage alone does not explain the pattern of disease recurrence in families (Brown et al. 2000*b*). Since familial AS is in nearly all cases restricted to those carrying *HLA-B27,* it has been postulated that *HLA-B27* is almost essential but not adequate to cause AS and that other genes interact with *HLA-B27* to cause the condition (Brown et al. 2002). Although several areas of suggestive or significant linkage have been reported from whole-genome scans, only one non-MHC gene, *CYP2D6* (MIM 124030) on chromosome 22, has had replicated association reported with AS (Beyeler et al. 1996; Brown et al. 2000*a*).

The chromosome 2q13 region was linked to AS in our previously reported whole-genome screen (Laval et al. 2001), with a maximum LOD score of 2.5 at a position 132 cM from the p-telomere. The magnitude of the sibling recurrence risk due to this locus is $\lambda = 1.7$ (95% CI 1.3–2.3), equivalent to 12% of the familiality of AS. The interleukin 1 (IL-1) family gene cluster lies 123–126 cM from the p-telomere. Association of alleles of the *IL-1RN* (MIM 147679) variable number of tandem repeats (VNTR) with AS has been reported in some studies (McGarry et al. 2001; van der Paardt et al. 2002) but not others (Djouadi et al. 2001). Maksymowych et al. (2003) demonstrated association between AS and SNPs and SNP haplotypes within IL-1RN.

The prototypic members of the IL-1 family gene cluster are the genes *IL-1A* (MIM 147760), *IL-1B* (MIM 147720), and *IL-1RN*. *IL-1A* and *-B* encode proinflammatory cytokines involved in host defense against infection. The IL-1 receptor antagonist IL-1RA, encoded by the gene *IL-1RN*, is an anti-inflammatory nonsignalling molecule that competes for receptor binding with IL-1α and IL-1β. Six genes with structural homology to *IL-1A/B* or *IL-1RN* lie between *IL-1A* and

Received January 20, 2004; accepted for publication July 20, 2004; electronically published August 12, 2004.

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0002-9297/2004/7504-0006$15.00

IL-1RN. They are named *IL-1F5* (MIM 605507), *IL-1F6* (MIM 605509), *IL-1F7* (MIM 605510), *IL-1F8* (MIM 605508), *IL-1F9* (MIM 605542), and *IL-1F10*; *IL-1A, IL-1B,* and *IL-1RN* have been renamed *IL-1F1, IL-1F2,* and *IL-1F3,* respectively (Sims et al. 2001). The genes are ordered, from centromere to telomere, as *IL-1A, IL-1B, IL-1F7, IL-1F9, IL-1F6, IL-1F8, IL-1F5, IL-1F10, IL-1RN,* in a cluster within an ~360-kb region (Nicklin et al. 2002).

Given these findings and the prominent role of proteins encoded by genes lying within this complex in inflammation and in host defense against infection, we sought to test whether IL-1 complex genes were associated with AS. SNPs were identified in all nine IL-1 cluster gene members and, along with a VNTR in *IL-1RN,* were genotyped in families and unrelated controls. We demonstrate strong transmission disequilibrium of alleles and haplotypes of these markers in two separate family collections and demonstrate association by comparing cases from these families and unrelated healthy controls. Our findings strongly suggest that the IL-1 gene cluster contains a major susceptibility locus for AS.

Subjects, Material, and Methods

Families and Control Individuals

We studied 227 white British families containing affected sibling pairs and 317 parent-case trios with AS, as well as 200 healthy blood donors. AS was defined by the modified New York diagnostic criteria (van der Linden et al. 1984). The diagnosis of AS was confirmed, in all cases, by a qualified rheumatologist and was followed by either examination or telephone interview (conducted by L.B. or M.A.B.). In cases with an atypical history or no previous radiographic evidence, pelvic and lumbosacral spine radiographs were obtained and attending general practitioners were contacted to confirm diagnosis. All cases were HLA-B27 positive. There were 930 cases (66% male), who were, on average, 43.9 years of age and had been affected for 21.6 years. The study was approved by the Multicentre Research Ethics Committee, and all participants gave informed written consent.

SNP Identification and Selection

We identified SNPs in the IL-1 gene cluster by using a combination of literature searches and public SNP databases. *IL-1A*, *IL-1B,* and *IL-1RN* have previously been extensively studied for polymorphisms and were therefore not sequenced further. For the remaining six genes, SNPs in exons, in exon-intron boundaries, or in the 5′ UTR of each gene were identified from SNP databases and then were verified by direct sequencing in 48 healthy controls. An EST lying between *IL-1F7* and *IL-1F9* (locus 129359) was also studied. Because of strong linkage disequilibrium ($D' > 0.9$) with other *IL-1RN* markers, the SNPs *IL-1RN8061* and *IL-1RN9589* were not analyzed further. Twenty-five SNPs with minor-allele frequencies $\geqslant 5\%$ were used for the subsequent studies, in addition to the *IL-1RN* VNTR.

Genotyping Methods

SNPs were genotyped either by Sequenom MassArray (Sequenom) or PCR/restriction digestion. The SNPs genotyped and the respective genotyping methods are given in table 1. Alleles of the *IL-1RN* 46-bp VNTR were characterized by PCR and separation by electrophoresis on 2% agarose gels.

Statistical Analysis

Results were subjected to rigorous quality control. All markers were genotyped in duplicate, and only consistent genotypes were accepted. In the multicase and single-case families, Mendelian inheritance was checked using the program GAS version 2 (A. Young, unpublished software). MERLIN (Abecasis et al. 2002) was used to identify unlikely recombination events suggestive of genotyping error; such genotypes were repeated and rejected if the experimental data were still equivocal. Genotype and allele frequencies were compared in the probands of the multicase and single-case families. Where these differed by >10%, the marker concerned was regenotyped.

Initial analysis consisted of examination of within-family association of single markers and two-marker haplotypes of contiguous polymorphisms in parent-case and affected-sibling-pair families, using TRANSMIT (Clayton and Jones 1999). *P* values were determined by bootstrap simulation, as implemented within TRANSMIT, to account for the linkage component when using multicase families; significance levels therefore reflect association rather than linkage (Clayton and Jones 1999). For each analysis, 10^6 simulations were performed, so that the minimum *P* value reported is $P < 10^{-6}$, which we have recorded as $P = 0$. The TRANSMIT bootstrap simulation method is described at the TRANSMIT Web site.

To provide further support for these findings, a case-control analysis was then performed, comparing allele and genotype frequencies in probands from the affected-sibling-pair families and parent-case trio families with findings in unrelated healthy control individuals. In this analysis, all markers were tested individually; specific two-marker haplotypes were also assessed, as outlined in the "Results" section. Haplotypes in unrelated control samples were determined using the program PHASE (Stephens and Donnelly 2003), a Bayesian haplotype reconstruction method; haplotypes were included in fur-

Table 1

Markers Studied and the Genotyping Methods Employed

Marker	SNP ID	Genotyping Method	Primer[a]		Enzyme/Extension Primer
			Forward	Reverse	
IL-1A559	3783559	Digest	tggtctctcatttgtctcatc	tgttgcttgctcagtgttttg	*Mse*I
IL-1A376	2071376	Digest	ccaagatgaagaccaaccag	aggtcaggcagggaaaggaa	*Bst*Y1
IL-1A889	1800587	Digest	ctttaataatagtaaccaggcaaga	gcccaaggtgtgtcttctgt	*Dpn*II
IL-1B3953	1143634	Digest	gtaaataggctgaaaggggaaa	gtaaataggctgaaaggggaaa	*Taq*I
IL-1B5810	1143633	Digest	gtatatgctcaggtgtcctc	catggagaattagcaagctg	*Fnu*4HI
IL-1B−511	16944	Digest	acttaagtttaggaatcttcccact	gagcttatctccagggttgc	*Ava*I
IL-1F7-1	2723187	Sequenom	acgttggatggcattagcctcatccttgag	acgttggatgaaactccccttttagagaccc	ctctgcggagaaaggaagtc
IL-1F7-2	2708947	Sequenom	acgttggatgtcttttatagggctcaggtg	acgttggatgaattgcaggaggtgcagatg	gggctcaggtgggctcc
LOCUS 129359	2708954	Digest	agggctcactcgaatgactt	atggggtaggaggagtgacc	*Bsp*HI
IL-1F9-1	2121334	Sequenom	acgttggatgagccagattgcaaagactag	acgttggatgggtgaagtgaacattccctg	tgcatggacatctacaaatggc
IL-1F6-1	1446512	Sequenom	acgttggatgtctagagtccattgaggtgg	acgttggatgatgctgcttctccatggaag	gagctggctttgctacagtaa
IL-1F6-2	1446511	Sequenom	acgttggatgagcttctcagagccactgtg	acgttggatgtcaggagctggctttgctac	gcatgctgcttctccatgg
IL-1F6-3	895497	Sequenom	acgttggatgcacacccgatgattgatatc	acgttggatgtgactttctcaacagcattg	tgatatcctgaatgctcccc
IL-1F8-1	1562304	Sequenom	acgttggatggtggaaaagaggcttgttag	acgttggatgctctcctgttacacactcag	gaggcttgttagagaggagg
IL-1F8-2	2197578	Digest	ggactgacagtggagcaaca	ggcctaacatcagatgcaaa	*Bsm*I
IL-1F8-3	1900287	Sequenom	acgttggatgctacactgtgcaacttcagc	acgttggatggggaaaatcatctgcatggg	cttcagcctgcccacctta
IL-1F5-1	990524	Sequenom	acgttggatgaagaaggaagaaagggaggg	acgttggatgtcagaccctaagagtcagtg	gaggcagggaggaaagaaagt
IL-1F5-2	2441375	Digest	ggaaattgtgaccagcacct	gagctctagactgggaggttcaatt	*Mfe*I
IL-1F5-3	1530548	Digest	gtaagctcacccactctgtgcccgt	acatgccagtgggtggac	*Rsa*I
IL-1F5-4	1374286	Sequenom	acgttggatggtgggcaggtgcaaactatt	acgttggatgattgttggacctcctgcttg	tgcaaactattttagagagggac
IL-1F10-1	996878	Sequenom	acgttggatggtctctttataggcctgctc	acgttggatgcccaatctatcttggctcac	aggcctgctcatagcatgg
IL-1F10-2	1446522	Sequenom	acgttggatggaatctctggatcttgctcc	acgttggatgccttcatcattctcctgcac	tctggatcttgctccacagaa
IL-1F10-3	3811058	Digest	caaggtggtgattcagagca	gggaggaaagggaagaagag	*Bsm*AI
IL-1RN8006	419598	Digest	ttctatctgaggaacaaccaactagtagc	caccagacttgacacaggacaggca	*Hpa*II
IL-1RN8061[b]	423904	Digest	acacaggaaggtgccaagca	tgcagacagacgggcaaagt	*Mwo*I
IL-1RNVNTR		Digest	ctcagcaacactcctat	tcctggtctgcaggtaa	46-bp repeat
IL-1RN9589[b]	454078	Digest	ttgtggggaccaggggagat	agcctggcactctgctgaat	*Ssp*I
IL-1RN11100	315952	Digest	agggaggcagcacaggactt	agtccctgcagtccttgcca	*Msp*AI

[a] Underlined bases are deliberate mismatches.

[b] Studied in AS family probands only; not assessed further because of strong linkage disequilibrium with other IL-1RN markers ($D' > 0.9$).

ther analysis if they were assigned >90% certainty. For case-control haplotype comparisons, haplotypes were determined in the parent-case trio and affected-sibling-pair families through use of the program GENEHUNTER and proband haplotypes (i.e., one per family) used for the cases (Kruglyak et al. 1996). Pairwise linkage disequilibrium was calculated using the program GOLD, through use of haplotype data from the healthy controls (Abecasis and Cookson 2000).

Having demonstrated significant association across multiple polymorphisms in linkage disequilibrium with one another, we employed stepwise conditional logistic regression (Cordell and Clayton 2002) and the conditional extended transmission/disequilibrium test (CETDT) (Koeleman et al. 2000) to determine whether one or more SNPs could be identified as the primary associated variant(s). This analysis was performed using all members of the parent-case trio and affected-sibling-pair families. These methods use a conditional logistic regression approach to investigate the interdependence of associated physically linked markers. Markers are entered or deleted from the conditional logistic regression equation in a stepwise manner, to determine whether a marker is important once the effects of other markers have already been accounted for. Phase-known genotype and haplotype relative risks (HRRs) can also be estimated without bias when this approach is used (Cordell and Clayton 2002). The CETDT can be considered to be a special case of the conditional logistic regression in which the effect of alleles is assumed to be multiplicative. The CETDT has the advantage of allowing for missing parental genotypes via an EM algorithm, but this utility can be prohibitively slow to implement when more than a few markers are being considered simultaneously. Also, the CETDT does not correct for any nonindependence between multiple affected offspring from the same family, which is accounted for within the stepwise conditional logistic regression method by use of robust Huber-White information sandwich variance estimation, allowing all affected individuals to be studied within multicase families.

Results

Within-family studies demonstrated significant association of both single-marker and two-marker haplotypes for several markers spread across the *IL-1* gene cluster

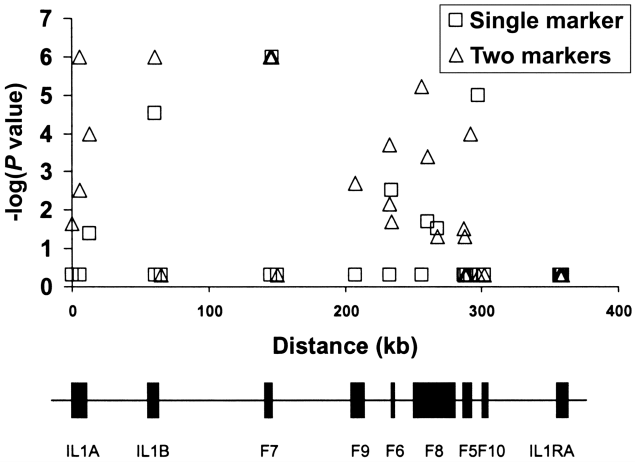

Figure 1 Single-marker and two-marker haplotype TDT findings obtained using parent-case trios and multicase families.

(see table 2). Seven markers achieved significance levels of $P < .05$ by within-family association. Two markers were sufficiently significant to remain so once Bonferonni correction was made for multiple (i.e., 26) comparisons (IL-1B−511, $P = .00003$; IL-1F10-3, $P = .00001$). Two-marker haplotypes gave the strongest associations, with 18 markers achieving $P < .05$, 6 of which achieved $P < 10^{-6}$ (see fig. 1 and table 2).

Case-control analysis confirmed either genotypic or allelic association for 10 markers (see table 3), all of which had been associated with AS in the within-family analysis, either as individual markers or as part of a two-marker haplotype.

To determine which marker or combination of markers was primarily responsible for the observed associations, we employed a stepwise conditional logistic regression approach (Cordell and Clayton 2002) (results were similar when the CETDT was used [Koeleman et al. 2000]). At the first stage, each marker was entered in turn into the regression equation, giving a 1-df test for the effect at a marker without accounting for effects at any other markers. This test is equivalent to performing a transmission/disequilibrium test (TDT) analysis at each marker in turn, using all families for which there are complete genotype data at the marker. Five markers were associated with AS in this analysis (IL-1B3953, IL-1B−511, IL-1F6-2, IL-1F8-1, and IL-1F10-3; $P = .007$, .0009, .047, .013, and .005 respectively). Each of these five markers, in turn, was accounted for by including the marker in the regression equation, and the effect of adding a second marker to the regression equation was examined for all the remaining markers, to test for their interdependence. Markers IL-1B−511 and IL-1F10-3 remained significant when added as second markers in the regression equation, regardless of which other of the five loci had been included as the first marker. If both of these markers were accounted for, under the assumption of multiplicative effects of haplotypes and applying Bonferroni correction for the number of markers studied, no other marker showed significant association with AS. When multiplicative effects of haplotypes are assumed in families with both parents genotyped, the combination of IL-1B−511 and IL-1F10-3 is significantly associated with AS ($P = .0002$). HRRs, significance levels, and CIs are given in table 4. Once IL-1B−511 was accounted for, IL-1F10-3 remained significantly associated ($P = .002$), and vice versa ($P = .0008$). Since this analysis used only families with both parents genotyped, the program TRANSMIT was used to test the association of this marker combination in all available families (both parent-case trio and affected-sibling-pair families). This demonstrated highly significant association ($P = 1.7 \times 10^{-8}$). Further, when proband haplotypes established using the program GENEHUNTER and control haplotypes identified using the program PHASE were compared, sig-

Table 2

Within-Family TDT Results when Individual Markers and Two-Marker Haplotypes Are Used

Marker	One-Marker *P* Value	Two-Marker *P* Value[a]
IL-1A559	NS	.022
IL-1A376	NS	.003
IL-1A889	NS	0
IL-1B3953	.04	.0001
IL-1B5810	NS	0
IL-1B−511	.00003	0
IL-1F7-1	NS	NS
IL-1F7-2	NS	.0003
LOCUS 129359	NS	NS
IL-1F9-1	NS	NS
IL-1F6-1	NS	.002
IL-1F6-2	NS	.0002
IL-1F6-3	NS	.007
IL-1F8-1	.003	.02
IL-1F8-2	NS	.000006
IL-1F8-3	.02	.0004
IL-1F5-1	.03	.05
IL-1F5-2	NS	.03
IL-1F5-3	NS	NS
IL-1F5-4	NS	.05
IL-1F10-1	NS	NS
IL-1F10-2	NS	.007
IL-1F10-3	.003	NS
IL-1RN8006	NS	NS
IL-1RNVNTR	NS	NS
IL-1RN11100	NS	NS

NOTE.—NS = not significant.

[a] Two-marker haplotypes consist of contiguous markers; *P* values are given with the 5′ marker of the two markers concerned.

Table 3

Genotype and Allele Frequencies in AS Cases (Parent-Case Trios and Multicase Families) and Controls

Marker	No. (%) of Cases with					No. (%) of Healthy Controls with					*P* Value[a]		Odds Ratio
	Genotype			Allele		Genotype			Allele				
	1 1	1 2	2 2	1	2	1 1	1 2	2 2	1	2	Genotype	Allele	
IL-1A559	211 (53.7)	142 (36.1)	40 (10.2)	564 (71.8)	222 (28.2)	112 (65.1)	52 (30.2)	8 (4.7)	276 (80.2)	68 (19.8)	.016	.003	.63
IL-1A376	29 (6.6)	207 (46.9)	205 (46.5)	265 (30)	617 (70)	19 (11)	77 (44.8)	76 (44.2)	115 (33.4)	229 (66.6)			
IL-1A889	151 (41.6)	174 (47.9)	38 (10.5)	476 (65.6)	250 (34.4)	87 (51.5)	71 (42)	11 (6.5)	245 (72.5)	93 (27.5)		.025	
IL-1B3953	262 (54.6)	193 (40.2)	25 (5.2)	717 (74.7)	243 (25.3)	98 (52.1)	82 (43.6)	8 (4.3)	278 (73.9)	98 (26.1)			
IL-1B5810	48 (9.8)	241 (49.4)	199 (40.8)	337 (34.5)	639 (65.5)	9 (5)	82 (45.8)	88 (49.2)	100 (27.9)	258 (72.1)	.049	.023	1.36
IL-1B−511	211 (41.3)	256 (50.1)	44 (8.6)	678 (66.3)	344 (33.7)	76 (38.8)	86 (43.9)	34 (17.3)	238 (60.7)	154 (39.3)	.0038	.047	1.28
IL-1F7-1	449 (86.7)	66 (12.7)	3 (0.6)	964 (93.1)	72 (6.9)	147 (88.6)	18 (10.8)	1 (0.6)	312 (94)	20 (6)			
IL-1F7-2	447 (84.5)	77 (14.6)	5 (0.9)	971 (91.8)	87 (8.2)	161 (83)	32 (16.5)	1 (0.5)	354 (91.2)	34 (8.8)			
LOCUS 129359	415 (85.4)	67 (13.8)	4 (0.8)	897 (92.3)	75 (7.7)	164 (85.9)	26 (13.6)	1 (0.5)	354 (92.7)	28 (7.3)			
IL-1F9-1	358 (68.8)	151 (29)	11 (2.1)	867 (83.4)	173 (16.6)	132 (72.1)	44 (24)	7 (3.8)	308 (84.2)	58 (15.8)			
IL-1F6-1	220 (46)	225 (47.1)	33 (6.9)	665 (69.6)	291 (30.4)	103 (56.3)	66 (36.1)	14 (7.7)	272 (74.3)	94 (25.7)	.037		.79
IL-1F6-2	231 (45.2)	253 (49.5)	27 (5.3)	715 (70)	307 (30)	94 (55)	69 (40.4)	8 (4.7)	257 (75.1)	85 (24.9)			
IL-1F6-3	262 (51.8)	217 (42.9)	27 (5.3)	741 (73.2)	271 (26.8)	109 (68.1)	41 (25.6)	10 (6.3)	259 (80.9)	61 (19.1)	.0005	.005	.64
IL-1F8-1	400 (84.2)	74 (15.6)	1 (0.2)	874 (92)	76 (8)	172 (88.2)	23 (11.8)	0 (0)	367 (94.1)	23 (5.9)			
IL-1F8-2	214 (43.9)	235 (48.3)	38 (7.8)	663 (68.1)	311 (31.9)	99 (53.5)	69 (37.3)	17 (9.2)	267 (72.2)	103 (27.8)	.039		.82
IL-1F8-3	186 (43.6)	209 (48.9)	32 (7.5)	581 (68)	273 (32)	102 (52.3)	79 (40.5)	14 (7.2)	283 (72.6)	107 (27.4)			
IL-1F5-1	184 (44.7)	193 (46.8)	35 (8.5)	561 (68.1)	263 (31.9)	105 (53.6)	79 (40.3)	12 (6.1)	289 (73.7)	103 (26.3)		.045	
IL-1F5-2	62 (14.8)	216 (51.4)	142 (33.8)	340 (40.5)	500 (59.5)	52 (28.4)	70 (38.3)	61 (33.3)	174 (47.5)	192 (52.5)	.0002	.023	.75
IL-1F5-3	82 (19.5)	201 (47.7)	138 (32.8)	365 (43.3)	477 (56.7)	20 (11.3)	82 (46.3)	75 (42.4)	122 (34.5)	232 (65.5)	.017	.004	1.46
IL-1F5-4	158 (37.7)	211 (50.4)	50 (11.9)	527 (62.9)	311 (37.1)	69 (37.7)	86 (47)	28 (15.3)	224 (61.2)	142 (38.8)			
IL-1F10-1	413 (82.9)	79 (15.9)	6 (1.2)	905 (90.9)	91 (9.1)	156 (83.4)	28 (15)	3 (1.6)	340 (90.9)	34 (9.1)			
IL-1F10-2	355 (77)	101 (21.9)	5 (1.1)	811 (88)	111 (12)	147 (80.8)	30 (16.5)	5 (2.7)	324 (89)	40 (11)			
IL-1F10-3	404 (90.6)	40 (9)	2 (0.4)	848 (95.1)	44 (4.9)	170 (90.4)	18 (9.6)	0 (0)	358 (95.2)	18 (4.8)			
IL-1RNVNTR	256 (53.3)	195 (40.6)	29 (6)	707 (73.6)	253 (26.4)	89 (53.3)	70 (41.9)	8 (4.8)	248 (74.3)	86 (25.7)			

[a] Significant *P* values ($P \leq .05$) are listed for alleles and genotypes.

Table 4

HRRs for Combination of SNPs IL-1B−511 and IL-1F10-3 in Parent-Case Trios, Relative to Haplotype 2-2

Haplotype	HRR	95% CI	*P* Value
1-1	11.3	2.6–49.8	.001
1-2	8.7	1.9–39.1	.005
2-1	5.8	1.3–27.5	.028

nificant association of this haplotypes was also observed ($P = .04$).

Linkage disequilibrium across the cluster was quite variable and appears to form three main blocks (see table 5). Moderate linkage disequilibrium was observed between IL-1B−511 and IL-1F10-3 ($D' = 0.27$; $P = .01$).

Discussion

These results strongly indicate that a gene or combination of genes within the IL-1 gene cluster significantly influence susceptibility to AS. Several genetic studies have hinted at association of the IL-1 region with AS and, in particular, with polymorphisms of *IL-1RN*. Two groups have previously reported association between allele 2 of a VNTR in the *IL-1RN* gene and AS (McGarry et al. 2001; van der Paardt et al. 2002). This polymorphism is associated with lower levels of IL-1RA production. Recently, Maksymowych and colleagues reported strong association of haplotypes of three SNPs within the 3′ region of the *IL-1RN* gene with AS, but they did not study the *IL-1RN* VNTR (Maksymowych et al. 2003). The fact that the haplotypic findings were stronger than individual SNP associations suggests either that these markers were not themselves involved in AS or that they acted in combination. Our findings do not demonstrate association of the *IL-1RN* VNTR with AS, nor do they demonstrate association of the other SNPs or haplotypes of polymorphisms within *IL-1RN* with the disease, making it unlikely that variation in this gene is responsible for the strong association we saw elsewhere in this region. Previous positive findings at this locus may be due to linkage disequilibrium with the associated haplotypes that we report here. Extensive linkage disequilibrium was seen across the IL-1 gene cluster (see fig. 2), and, thus, associations may be seen at loci physically quite remote from the true associated variant(s).

The genes *IL-1A, IL-1B,* and *IL-1RN* have been extensively sequenced in the past, and comprehensive data on their polymorphisms are available in public databases (University of Washington–Fred Hutchinson Cancer Research Center [UW-FHCRC] Variation Discovery Resource Web site). Much less is known about genes *IL-1F5–F10,* including information about polymorphisms, expression patterns in tissues and disease, or function. The different IL-1 family members signal through a range of IL-1 receptors, with IL-1α/β signalling through IL-1R1 and IL-1R2, IL1-F6 signalling through IL-1R6, and IL-18 signalling through IL-1R5. IL-1F5 inhibits IL-1F6 activity, and IL-1F7 inhibits IL-18 activity. IL-1F8, -9, and -10 are thought to be IL-1 antagonists, since their sequences are most similar to IL-1RN, but their exact function is undetermined. IL-1F10 binds IL-1R1, suggesting that it may play a role in regulating IL-1A/B function (Smith et al. 2000). Although it appears that IL-1α, IL-1β, and IL-1RA are expressed at greater levels than other IL-1 gene family members, they are all widely expressed, including on activated monocytes and B cells (Laurincova 2000; Smith et al. 2000)

Very little is known about the role of IL-1 in ankylosing spondylitis. Using in situ hybridization methods, Braun et al. (1995) found no IL-1 mRNA in sacroiliac joint biopsy specimens from three patients with AS. There are contradictory findings regarding serum IL-1β levels, with studies showing either increased IL-1β levels in serum from patients with AS compared with healthy controls (Vazquez-Del et al. 2002) or no difference compared with controls with noninflammatory back pain (Gratacos et al. 1994). Stimulation of blood cell cultures with the superantigen *Mycoplasma arthritidis* supernatant suggested that AS cases had impaired IL-1B production (Brand et al. 1997). Expression patterns of other IL-1 family members in AS have not been reported.

Our results point to the presence of one or more susceptibility loci within the IL-1 complex, marked by the IL-1B−511 and IL-1F10-3 polymorphisms. Linkage disequilibrium is significant across the entire IL-1 gene cluster, perhaps explaining the broad area across which we observed association. Conditional logistic regression analysis demonstrated that IL-1B−511 and IL-1F10-3 were consistently associated with disease even when the influence of other associated markers was controlled for. Haplotypes of these two markers were strongly associated with disease both within families ($P = .0002$ in the fully typed parent-case trio and affected-sib-pair families; $P = 1.7 \times 10^{-8}$ among all families) and by case-control analysis ($P = .04$). Significant residual association was noted when either of these markers was accounted for, suggesting either that there is one locus with a susceptibility allele that lies on more than one haplotype or that there is more than one susceptibility locus for AS within the IL-1 family gene cluster. The fact that the associations observed were strongest for haplotypes rather than individual SNPs suggests that the markers reported here either are not themselves causally associated with AS or encode only part of the

Table 5

Pairwise Linkage Disequilibrium Values across the IL-1 Gene Cluster

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 (IL-1A559)		0	0	0	0	.25	.53	.26	.22	0	.14	.05	.08	.08	0	0	0	.23	.46	0	0	0	0	0	.57	.01	.91	0
2 (IL-1A376)	.81		0	0	.96	.02	.69	.19	.39	0	0	0	0	.01	0	0	0	.01	.30	0	0	0	0	.02	.13	0	.58	.02
3 (IL-1A889)	.59	.80		0	0	.31	.95	.42	.20	0	0	0	0	.37	0	0	0	0	.02	0	0	0	.04	0	.03	0	.01	0
4 (IL-1B3953)	.49	.73	.57		0	0	.01	0	0	0	.01	.01	.01	.02	0	.01	.02	0	0	0	0	0	0	0	.02	0	.42	0
5 (IL-1B5810)	.48	0	.52	.86		0	0	0	0	0	.02	0	.01	.15	0	0	.01	0	0	0	0	0	0	0	.02	0	.02	.48
6 (IL-1B−511)	.05	.21	.04	.20	.79		.11	.24	.29	0	.59	.11	.29	.79	.01	0	.01	0	0	.04	0	0	.01	0	0	0	0	.35
7 (IL-1F7-1)	.05	.08	.01	.21	.68	.14		0	0	.83	.67	.60	.79	0	.60	.17	.26	0	0	.37	.03	.05	0	.01	.17	.49	.93	.39
8 (IL-1F7-2)	.09	.25	.07	.24	.70	.10	.94		0	.31	.50	.87	.94	0	.48	.07	.19	0	0	.47	.02	.04	0	.03	.48	.40	.96	.17
9 (LOCUS 129359)	.11	.18	.12	.30	.59	.10	.83	.90		.32	.73	.88	.63	0	.82	.26	.80	0	0	.46	.01	.02	0	.01	.10	.55	.52	.08
10 (IL-1F9-1)	.47	.77	.48	.66	.83	.23	.01	.06	.06		.73	.86	.54	.12	.73	.33	.70	0	0	.06	0	0	0	0	.01	.01	.45	.09
11(IL-1F6-1)	.06	.76	.16	.11	.20	.02	.03	.05	.03	.05		0	0	0	0	0	0	.21	.66	0	.13	.04	.01	.78	.32	.02	.19	.84
12 (IL-1F6-2)	.08	.69	.16	.12	.24	.07	.04	.01	.01	.02	.95		0	0	0	0	0	.08	.56	0	.07	.04	0	.80	.43	.02	.28	.83
13 (IL-1F6-3)	.08	.73	.18	.11	.22	.05	.02	.01	.04	.09	.94	.94		0	0	0	0	.07	.66	0	.03	0	0	.74	.35	.03	.30	.48
14 (IL-1F8-1)	.16	.58	.09	.19	.25	.05	.18	.17	.19	.10	.65	.64	.62		0	0	0	.23	.98	0	.01	.01	0	.02	.38	0	.27	0
15 (IL-1F8-2)	.13	.74	.18	.15	.27	.11	.05	.06	.02	.02	.70	.68	.73	.65		0	0	.45	.01	0	.21	.66	.07	.32	.01	.01	.02	.48
16 (IL-1F8-3)	.15	.76	.21	.12	.23	.12	.12	.15	.10	.06	.68	.64	.71	.65	.92		0	.39	0	0	.45	.83	.03	.30	.01	.01	.06	.93
17 (IL-1F5-1)	.13	.72	.18	.10	.20	.11	.10	.11	.02	.02	.66	.65	.68	.62	.91	.89		.13	0	0	.04	.09	.28	.34	0	.03	.02	.57
18 (IL-1F5-2)	.09	.14	.21	.40	.36	.59	.67	.62	.73	.59	.10	.13	.14	.19	.04	.04	.07		0	.59	0	0	.14	0	0	0	.06	.25
19 (IL-1F5-3)	.05	.06	.15	.38	.41	.58	.75	.69	.68	.50	.03	.04	.03	0	.13	.14	.18	.80		.01	0	0	.15	0	0	0	.03	.05
20 (IL-1F5-4)	.26	.72	.27	.29	.25	.09	.09	.07	.08	.13	.69	.68	.71	.58	.91	.85	.90	.02	.12		.18	.38	.10	.37	.01	.05	.01	0
21 (IL-1F10-1)	.33	.70	.40	.62	.74	.24	.10	.10	.13	.68	.10	.13	.15	.14	.20	.12	.32	.90	.93	.19		0	0	.02	.66	.93	.71	0
22 (IL-1F10-2)	.31	.63	.35	.55	.69	.23	.09	.09	.12	.65	.14	.14	.20	.13	.07	.03	.26	.91	.88	.12	.97		0	.06	.61	.88	.66	0
23 (IL-1F10-3)	.53	.81	.23	.33	.63	.27	.20	.19	.20	.31	.25	.35	.29	.24	.20	.24	.12	.28	.28	.21	.24	.23		0	0	.01	0	.05
24 (IL-1RN8006)	.16	.28	.18	.21	.50	.44	.17	.14	.18	.14	.01	.03	.04	.17	.11	.11	.10	.47	.57	.08	.14	.11	.48		0	0	0	.09
25 (IL-1RN8061)	.02	.06	.10	.09	.19	.31	.11	.05	.14	.13	.10	.08	.10	.07	.26	.24	.31	.40	.51	.20	.03	.03	.36	.62		0	0	.35
26 (IL-1RNVNTR)	.10	.31	.18	.17	.53	.46	.05	.06	.04	.15	.10	.09	.08	.07	.11	.12	.08	.63	.75	.11	.02	.03	.30	.74	.52		0	0
27 (IL-1RN9589)	.01	.06	.12	.03	.20	.25	.01	0	.13	.04	.13	.11	.11	.24	.22	.17	.21	.14	.16	.20	.03	.03	.40	.44	.45	.33		.03
28 (IL-1RN11100)	.17	.24	.13	.17	.03	.08	.07	.11	.15	.09	.02	.02	.07	.53	.03	.01	.03	.06	.10	.21	.21	.20	.20	.20	.09	.32	.22	

NOTE.—Numbers (1–28) correspond to the same marker names in both the columns and rows. Lewontin's standardized linkage disequilibrium coefficient is given in the bottom left half of the table, and the corresponding P values are given in the upper right of the table. P values <.001 are reported as $P = 0$.

susceptibility effect demonstrated in this region. We have initiated further mapping studies to identify the true disease-causing variants, which could have significant implications for the development of novel therapeutic targets.

Acknowledgments

This work was funded by the Arthritis Research Campaign (United Kingdom). The authors would like to thank the family members who participated in the study and the National Ankylosing Spondylitis Society (United Kingdom) for their support of this work.

Electronic-Database Information

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for AS, *CYP2D6*, and genes within IL1 gene cluster)

TRANSMIT, http://www-gene.cimr.cam.ac.uk/clayton/software/ (for TRANSMIT software and description of the bootstrap simulation method)

UW-FHCRC Variation Discovery Resource, http://pga.gs.washington.edu/finished_genes.html

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