

Interleukin 1 and Nuclear Factor- κ B Polymorphisms in Ankylosing Spondylitis in Canada and Korea

TAE-HWAN KIM, MILLICENT A. STONE, PROTON RAHMAN, DAE-HYUN YOO, YONG-WOOK PARK, URSULA PAYNE, DAVE HALLETT, and ROBERT D. INMAN

ABSTRACT. Objective. The interleukin 1 α and 1 β (IL-1 α , IL-1 β) are potent mediators of inflammation and immunity. IL-1 receptor antagonist (IL-1Ra) is a protein that binds to IL-1 receptors and competitively inhibits the binding of IL-1 α and IL-1 β . There are reports of IL-1 complex gene polymorphisms in ankylosing spondylitis (AS), but the results have been inconsistent. NFKB1 encodes the genes for the p50 and p101 nuclear factor- κ B (NF- κ B) isoforms, which are recognized as critical to inflammatory disease. To date there have been no reports examining an association between NFKB1 and AS. We investigated polymorphisms of IL-1 complex and NF- κ B1 with 2 genetically and geographically different populations.

Methods. Subjects with AS satisfied modified New York criteria for AS. Healthy controls were recruited at each respective site. Subjects with AS were genotyped for the following: IL-1 α -889 single nucleotide polymorphism (SNP); IL-1 β +3953 SNP; IL-1Ra (86 base pair variable number tandem repeat within intron 2); and NFKB1 (-94 insertion/deletion polymorphism).

Results. In total, 205 subjects with AS and 200 controls from Seoul, Korea, and 68 subjects with AS and 164 controls from Toronto, Canada, were genotyped for the IL-1 α and IL-1 β polymorphisms and 115 controls for the IL-1Ra and NF- κ B polymorphisms. There were no differences of IL-1 α , IL-1 β , IL-1Ra, and NF- κ B polymorphisms between AS patients and controls in these populations.

Conclusion. Our analysis of these SNP in the IL-1 complex and NF- κ B genes does not support a major role for either in AS susceptibility in the Seoul and Toronto populations. (J Rheumatol 2005;32:1907-10)

Key Indexing Terms:

INTERLEUKIN 1 α
NUCLEAR FACTOR κ B

INTERLEUKIN 1 β

RECEPTOR ANTAGONIST
ANKYLOSING SPONDYLITIS

Ankylosing spondylitis (AS) is the prototype of the spondyloarthropathies (SpA) and is characterized by inflammation of spine and sacroiliac joints. Although HLA-27 is the dominant gene with respect to AS susceptibility, whole-genome screening in western populations with AS has implicated non-MHC genes in conferring susceptibility¹.

The interleukin 1 (IL-1) family consists of 3 homologous proteins: IL-1 α , IL-1 β , and IL-1 receptor antagonist (IL-

1Ra). These are encoded by the genes IL-1A, IL-1B, and IL-1RN, respectively². The IL-1 α and IL-1 β proteins are synthesized by a variety of cell types including activated macrophages, stimulated B lymphocytes, and fibroblasts, and are potent mediators of inflammation and immunity. The IL1RN protein binds to the IL-1 receptor and competitively inhibits the binding of IL-1 α and IL-1 β . As a consequence, the biologic activity of these 2 cytokines can be neutralized in physiologic and pathophysiologic inflammatory responses². The IL-1 family gene cluster is located on chromosome 2q13, and this region shows strong linkage in genome-wide screens¹. There are increased IL-1 β levels in serum from patients with AS, and AS patients exhibit diminished IL-1 β production after mycoplasma infection^{3,4}. There are some reports that polymorphisms of IL-1Ra gene, not IL-1 α /IL-1 β , are associated with inflammatory diseases including AS⁵⁻⁷.

Nuclear factor- κ B (NF- κ B) is an inducible transcription factor that regulates expression of key cytokines involved in the pathogenesis of inflammatory joint diseases. Recently, the first potentially functional polymorphism of the NFKB1 gene was described⁸: an ATTG insertion/deletion polymorphism that was associated with ulcerative colitis (UC). Given that an association has been reported several times

From the Hospital for Rheumatic Diseases, Hanyang University, Seoul, Korea; St. Michael's Hospital, Toronto, Ontario, Canada; Memorial University of Newfoundland, St. John's, Newfoundland, Canada; and Toronto Western Hospital and Mount Sinai Hospital, University of Toronto, Toronto, Canada.

T-H. Kim, MD, PhD, Assistant Professor; D-H. Yoo, MD, PhD, Professor of Medicine; Y-W. Park, MD, PhD, Clinical Fellow, Hanyang University; M.A. Stone, MD, MSc, Assistant Professor of Medicine, University of Toronto, St. Michael's Hospital; P. Rahman, MD, Associate Professor of Medicine, Memorial University; U. Payne, BSc, Research Associate, Toronto Western Hospital Research Institute; D. Hallett, MSc, Biostatistician, Mount Sinai Hospital, University of Toronto; R.D. Inman, MD, Professor of Medicine, University of Toronto.

Address reprint requests to Dr. R.D. Inman, The Arthritis Center of Excellence, Toronto Western Hospital, ECW 8-005, 399 Bathurst Street, Toronto, Ontario M5T 2S8, Canada. E-mail: Robert.Inman@uhn.on.ca

Accepted for publication May 30, 2005.

between UC and polymorphisms in the tumor necrosis factor- α (TNF- α) gene⁹, and that TNF- α is involved with induction of NF- κ B production and TNF- α is the most important cytokine in the pathogenesis of AS, we investigated polymorphisms of the IL-1 family and NF- κ B promoter in 2 AS populations differing widely both geographically and ethnically.

MATERIALS AND METHODS

Patients. Patients with AS were drawn from the Spondylitis Clinics at the Hospital for Rheumatic Diseases, Hanyang University, Seoul, and the Toronto Western Hospital, University of Toronto. All patients satisfied the modified New York criteria for the diagnosis of AS¹⁰. Controls were from the same ethnic background of both areas and were healthy at the time blood samples were obtained. The study was approved by the respective regional ethics committees, and written informed consent was obtained from all study participants.

Genotyping. IL-1 α , IL-1 β , NFKB1. DNA samples were genotyped for 2 IL-1 variants and NF- κ B by time-of-flight mass spectrometry using the Sequenom platform (Table 1). First, 2.5 ng of genomic DNA was amplified under standard conditions using the forward and reverse primer pairs and reactions were multiplexed where possible. After DNA amplification, all unincorporated nucleotides in the polymerase chain reaction (PCR) product were deactivated using shrimp alkaline phosphatase. A primer extension reaction was then carried out using the mass-extend primer and the appropriate termination mix as indicated in Table 1. The primer extension products were then cleaned and spotted onto a SpectroChip. The chip was scanned using a mass spectrometry workstation (Bruker), and the resulting spectra were analyzed using the Sequenom SpectroTyper-RT software.

IL-1Ra. The following protocol was followed for DNA amplification: the PCR reaction mixture contained 1 \times PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°C), 1.5 mM MgCl₂, and 0.1% Triton X-100], 1.5 mM MgCl₂, 30 μ M each of dNTP, 6% DMSO, 1 μ M each forward and reverse primer, 0.4 units of Taq DNA Polymerase (Promega, Madison, WI, USA), and 100 ng of genomic DNA. The PCR was amplified in a DNA Engine Tetrad (MJ Research, Watertown, MA, USA) with an initial denaturation at 94°C for 2 min, followed by 40 cycles of 94°C denaturation for 60 s, 58°C annealing for 60 s, and 72°C extension for 90 s, and a final extension step at 72°C for 2 min. The PCR products were run on a 2% agarose gel containing ethidium bromide and were visualized with UV light.

Statistical analysis. Minor allele frequencies for cases and controls were assessed using the p value from the Wald chi-square statistics, and odds ratios and 95% confidence intervals were also based on the Wald tests.

RESULTS

In total, 405 subjects from Seoul were genotyped (205 patients with AS, 200 controls); 232 subjects from Toronto (68 patients with AS, 164 controls) were genotyped for the IL-1 α and IL-1 β polymorphisms, and 115 controls for the IL-1Ra and NF- κ B polymorphisms. In Seoul, patients and controls were exclusively native-born Koreans; 92% (n = 188) of the Korean AS patients were men. The mean age of the AS patients was 31.4 years and mean disease duration was 11.6 years. HLA-B27 was present in 99% (201/203); 58% had peripheral arthritis and 22% had a history of uveitis. There were no patients with inflammatory bowel disease (IBD) or psoriasis. In Toronto, only Caucasian subjects with AS were selected for study; 81% (n = 55) of the Canadian AS patients were men. The mean age of these patients was 39.3 years; 52% had peripheral arthritis and 22% had a history of uveitis. In the Canadian AS cohort, 21% had IBD and 9% had psoriasis. HLA-B27 was positive in 83%. In both centers, controls were healthy volunteers screened by questionnaire to exclude those with a personal or family history of arthritis.

There were no differences of IL-1 α , IL-1 β , IL-1Ra, and NFKB1 polymorphism in the 2 populations (Table 2). Compared to patients and controls in Seoul, Toronto subjects showed a higher frequency of IL-1 α , IL-1 β , and allele 2 frequency of IL-1Ra. Stratification by sex and B27 status did not affect the results.

DISCUSSION

Chromosome 2q13, on which the IL-1 family gene cluster lies, yields a strong signal in genome-wide screens of AS¹. Several polymorphisms of the IL-1 family cluster have been examined in inflammatory diseases including AS^{5-7,11,12}. These are position -889 of IL-1 α , positions -31, -511 and +3953 in IL-1 β , and some single nucleotide polymorphisms (SNP) and variable number tandem repeats (VNTR) on intron 2 of the IL-1RN gene. VNTR intron 2 of IL-1RN is associated with insulin-dependent diabetes mellitus¹³ and

Table 1. Oligonucleotide sequence for the IL-1 family and NFKB1.

Variation	Primer	Sequence
IL1- α -889	Forward	5'- ACG TTG GAT GTT GGG AGA AAG GAA GGC ATG -3'
RS1143634	Reverse	5' - ACG TTG GAT GTT CTA CCA CCT GAA CTA GGC -3'
	Extension	5'- TTT ACA TAT GAG CCT TCA ATG -3'
IL1 β +3953	Forward	5'- ACG TTG GAT GAG TGA TCG TAC AGG TGC ATC -3'
Rs1800587	Reverse	5' - ACG TTG GAT GGT GCT CCA CAT TTC AGA ACC -3'
	Extension	5'- CTC GTT ATC CCA TGT GTC -3'
NFKB1 -94	Forward	5'- ACG TTG GAT GTA GGG AAG CCC CCA GGA AG -3'
AF213884	Reverse	5' - ACG TTG GAT GAT GAC TCT ATC AGC GGC ACT -3'
	Extension	5'- CGC CTG CCG GGC CCA AT -3'
IL1Ra	Forward	5'- CTC AGC AAC ACT CCT AT-3'
AF387734	Reverse	5' - GCA GCA ATA ATG AAG AG -3'
	Product	2 repeats: 335 bp; 3 repeats: 421 bp
	Size	4 repeats: 507 bp; 5 repeats: 593 bp

Table 2. Minor allele frequencies for the IL-1 family and NF- κ B polymorphism in AS patients and controls of 2 populations.

	Patients	Seoul Controls	p	Patients	Toronto Controls	p
IL-1 α -889 T allele, %	9 [2]	9.9 [3]	0.6824	36.5 [10]	29.9 [13]	0.4458
OR (95% CI)		0.9030 (0.5537–1.4726)			1.34 (0.72–2.52)	
IL-1 β -3953 T allele, %	4.3 [0]	3.2 [1]	0.4474	26.9 [5]	21.3 [7]	0.4803
OR (95% CI)		1.3378 (0.6300–2.8406)			1.35 (0.68–2.70)	
IL-1Ra allele 2*, %	4.3 [1]	6.7 [1]	0.1427	34.1 [7]	27.8 [7]	0.5440
OR (95% CI)		0.6344 (0.3436–1.1710)			1.34 (0.71–2.51)	
NF- κ B ATTTG -94 deletion, %	44.8 [42]	43.2 [32]	0.6522	41.3 [11]	39.4 [13]	0.6522
OR (95% CI)		1.0666 (0.8058–1.4118)			1.10 (0.60–2.0)	

[Number of homozygotes observed for the minor allele.] * Allele 2 is 2-repeat intron 2 of IL-1RN gene.

ulcerative colitis¹⁴. The IL-1 α polymorphism is associated with psoriatic arthritis¹⁵ and juvenile chronic arthritis¹⁶. The association of VNTR with AS has been positively reported in European populations^{5,6}. In a UK study, no significant differences were seen at the polymorphic alleles in the IL-1 α and IL-1 β genes, but there was a significant increase in the carriage of VNTR allele 2 of IL-1RN in patients with AS compared with controls (16% vs 8%; $p = 0.01$)⁵. A study from The Netherlands showed the same result as the UK investigation⁶. But Djouadi, *et al* showed there was no association between IL-1 genes and AS¹⁷. A previous Canadian study analyzed SNP of IL-1RN instead of VNTR allele 2 of IL-1RN. The frequencies of allele C at SNP position 30735 allele and allele G at position 31017 in exon 6 were increased in AS patients compared to controls. A highly significant difference in the overall distribution of haplotype frequencies was also evident between cases and controls⁷. We analyzed C889T of IL-1 α , C3953T of IL-1 β , and 2 repeats of intron 2 (allele 2), but our findings indicated there was no difference between AS patients and controls in 2 different populations, consistent with the findings of Djouadi, *et al*¹⁷. Further SNP analysis of IL-1RN (reported to be associated with AS⁷) is still required.

The frequency of the IL-1Ra1 allele differs in different AS study populations. One report from Scotland⁵ showed that the frequency of 2 repeats of the 86 base pair length of IL-1RN is increased in AS patients compared with controls. Although the frequency of 2 repeats in subjects in Seoul was lower than in those in Toronto in both AS patients and controls, there were no differences in the 2 populations. This finding is compatible with a report by Pyo, *et al* from Korea¹⁸. The low numbers of patients with IBD and psoriasis precluded an analysis of subset association with these genetic markers. Our analysis also did not include stratification by disease severity as defined for functional status (by the Bath Ankylosing Spondylitis Functional Index, BASFI) or radiographic criteria, and future studies might address this aspect, although larger numbers of subjects may be required.

NF- κ B proteins are transcription factors that regulate

various biological defense processes and apoptosis. Although there are 5 members of NF- κ B, NFKB1 that encodes genes for the p50 and p101 NF- κ B isoforms is particularly important for inflammatory diseases⁸. In UC there is an association with a distinct promoter polymorphism that affects the ability to bind nuclear proteins⁸. Although whole-genome screening in AS has not revealed a linkage with chromosome 4q, where NFKB1 is located¹, because TNF- α has proved to be a critical cytokine and NF- κ B is important for regulation of TNF- α , we examined the possible role of NF- κ B in AS. We found no differences in 2 AS populations in the context of local controls.

For the IL-1 α -889 SNP, IL-1 β +3953 SNP, and NFKB1 (-94 insertion/deletion polymorphism), the genotypes in both the control groups showed no evidence of a departure from Hardy-Weinberg equilibrium. If we assume that there was a 10% difference in the allele frequency between cases and controls for both populations, then a total of 273 cases and 364 controls examined in the 2 populations would result in 80% power for our study¹⁹. As the IL-1Ra is an 86 base pair VNTR with at least 5 alleles, Hardy-Weinberg equilibrium will have very low power due to the large number of alleles and will be very sensitive to small cells. As a result this analysis is likely not appropriate in this circumstance.

Although the IL-1 family, as well as NF- κ B, is very important in inflammation, we found no association with selected SNP of these genes and AS, studying 2 very diverse AS cohorts.

REFERENCES

1. Laval SH, Timms A, Edwards S, et al. Whole-genome screening in ankylosing spondylitis: evidence of non-MHC genetic-susceptibility loci. *Am J Hum Genet* 2001;68:918-26.
2. Nicklin MJ, Weith A, Duff GW. A physical map of the region encompassing the human interleukin-1 α , interleukin-1 β , and interleukin-1 receptor antagonist genes. *Genomics* 1994;19:382-4.
3. Gu J, Marker-Hermann E, Baeten D, et al. A 588-gene microarray analysis of the peripheral blood mononuclear cells of spondyloarthropathy patients. *Rheumatology Oxford* 2002;41:759-66.
4. Brand JM, Neustock P, Kruse A, Alvarez-Ossorio L, Schnabel A, Kirchner H. Stimulation of whole blood cultures in patients with

- ankylosing spondylitis by a mitogen derived from *Mycoplasma arthritis* (MAS) and other mitogens. *Rheumatol Int* 1997;16:207-11.
5. McGarry F, Neilly J, Anderson N, Sturrock R, Field M. A polymorphism within the interleukin 1 receptor antagonist (IL-1Ra) gene is associated with ankylosing spondylitis. *Rheumatology Oxford* 2001;40:1359-64.
 6. van der Paardt M, Crusius JB, Garcia-Gonzalez MA, et al. Interleukin-1 β and interleukin-1 receptor antagonist gene polymorphisms in ankylosing spondylitis. *Rheumatology Oxford* 2002;41:1419-23.
 7. Maksymowych WP, Reeve JP, Reveille JD, et al. High-throughput single-nucleotide polymorphism analysis of the IL1RN locus in patients with ankylosing spondylitis by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. *Arthritis Rheum* 2003;48:2011-8.
 8. Karban AS, Okazaki T, Panhuysen CI, et al. Functional annotation of a novel NFKB1 promoter polymorphism that increases risk for ulcerative colitis. *Hum Mol Genet* 2004;13:35-45.
 9. Sashio H, Tamura K, Ito R, et al. Polymorphisms of the TNF gene and the TNF receptor superfamily member 1B gene are associated with susceptibility to ulcerative colitis and Crohn's disease, respectively. *Immunogenetics* 2002;53:1020-7.
 10. van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York Criteria. *Arthritis Rheum* 1984;27:361-8.
 11. Timms AE, Crane AM, Sims AM, et al. The interleukin 1 gene cluster contains a major susceptibility locus for ankylosing spondylitis. *Am J Hum Genet* 2004;75:587-95.
 12. Bidwell JL, Wood NA, Morse HR, Olomolaiye OO, Laundry GJ. Human cytokine gene nucleotide sequence alignments, 1998. *Eur J Immunogenet* 1998;25:83-265.
 13. Pociot F, Ronningen KS, Bergholdt R, et al. Genetic susceptibility markers in Danish patients with type 1 (insulin-dependent) diabetes — evidence for polygenicity in man. Danish Study Group of Diabetes in Childhood. *Autoimmunity* 1994;19:169-78.
 14. Mansfield JC, Holden H, Tarlow JK, et al. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology* 1994;106:637-42.
 15. Ravindran JS, Owen P, Lagan A, et al. Interleukin 1 α , interleukin 1 β and interleukin 1 receptor gene polymorphisms in psoriatic arthritis. *Rheumatology Oxford* 2004;43:22-6.
 16. McDowell TL, Symons JA, Ploski R, Forre O, Duff GW. A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1 α polymorphism. *Arthritis Rheum* 1995;38:221-8.
 17. Djouadi K, Nedelec B, Tamouza R, et al. Interleukin 1 gene cluster polymorphisms in multiplex families with spondylarthropathies. *Cytokine* 2001;13:98-103.
 18. Pyo CW, Hur SS, Kim YK, et al. Polymorphisms of IL-1B, IL-1RN, IL-2, IL-4, IL-6, IL-10, and IFN- γ genes in the Korean population. *Hum Immunol* 2003;64:979-89.
 19. Power analysis and sample size. PASS 2002. Kaysville, UT: NCSS Inc. Internet. Available from: <http://www.ncss.com/pass.html>. Accessed July 8, 2005.