Association of Nuclear Factor-κB in Psoriatic Arthritis

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ABSTRACT. ***Objective.*** To examine the association of single nucleotide polymorphisms (SNP) in the *NFKB1* gene, as well as 2 genes in the nuclear factor (NF)-κB functional complex (*RelA* and *NFKBIA*), in patients with psoriatic arthritis (PsA) from Newfoundland.

Methods. Patients with PsA and controls were genotyped for one 4-base insertion/deletion and 5 SNP in *NFKB1*, 4 SNP in *RelA*, and 7 SNP in *NFKBIA* by time-of-flight mass spectrometry, using the Sequenom platform. Chi-square analysis was used to test the single locus associations between SNP in the NF-κB complex and PsA. Associations between multi-locus haplotypes and case or control status were tested using the software PHASE.

Results. Two hundred and twenty-four patients with PsA (52% male) and 88 ethnically matched controls (64% male) were genotyped. No association was noted with any of the SNP tested for the single locus associations in *NFKB1*, *RelA*, and *NFKBIA* or with multi-locus haplotypes. In particular, the allele frequency for the *NFKB1* -94delATTG was 41.7% in cases and 41.6% in the controls ($p = 0.97$).

Conclusion. No association between the *NFKB1* -94 ins/delATTG promoter polymorphism or with other NF-κB complex SNP in patients with PsA from Newfoundland was observed. (J Rheumatol 2005;32:1742–4)

Key Indexing Terms:
PSORIATIC ARTHRITIS
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Psoriatic Arthritis (PsA) is a complex immunologically mediated disorder that results from interplay between multiple genetic and environmental factors. Epidemiological studies implicate a substantive role for genetic factors in disease susceptibility and expression in PsA[1,2]. Although association of PsA and alleles in the major histocompatibility complex (MHC) region is well established, this region appears to contribute only one-third of the total genetic variance to other forms of autoimmune inflammatory arthritis[3]. Thus, it is prudent to investigate high priority candidate genes outside the MHC region in PsA.

Nuclear factor-κB (NF-κB) is a pleiotropic multiprotein complex that regulates key cytokines including tumor necrosis factor (TNF)-α and interleukin (IL)-1ß involved in the immune response[4]. These cytokines are important in the pathogenesis of PsA[4]. Recently, the first potentially functional polymorphism of the *NFKB1* gene on chromosome 4q24 was described: an ATTG ins/del polymorphism in the promoter region of the gene that decreases promoter activity in ulcerative colitis[5]. NF-κB has also been implicated in the pathogenesis of rheumatoid arthritis (RA), as the affinity for NF-κB binding in RA synovium is significantly higher than in osteoarthritis synovium[6].

Based on the proposed function of *NFKB1* and its association with inflammatory arthritis and colitis, we investigated the role of this novel *NFKB1* promoter ins/del polymorphism as well as other single nucleotide polymorphisms (SNP) in the *NFKB1* gene, and 2 genes in the NF-κB functional complex (*RelA* and *NFKBIA*) in patients with PsA.

MATERIALS AND METHODS

Patients. This study was approved by the local ethics committee at Memorial University of Newfoundland. Informed consent was obtained from all patients, who were from Newfoundland. PsA was diagnosed as an inflammatory arthritis in patients with psoriasis in the absence of other etiologies for inflammatory arthritis. With respect to patterns of PsA in our cohort, 61.4% had polyarthritis, 33.6% oligoarthritis, 2.6% isolated spondyloarthropathy, and 1.3% had the distal interphalangeal (DIP) variant of PsA. Fifty-two percent of our cohort had nail changes associated with psoriasis, 35% had dactylitis, and 20% had tendinitis at study enrollment. Controls were also from Newfoundland and were unrelated to each other and to our patients.

Genotyping. Whole blood samples were obtained from patients with PsA

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and controls. DNA was extracted using the Promega Wizard Genomic DNA purification kit. Detection of SNP was performed by analysis of primer extension products generated from previously amplified genomic DNA using a chip-based MALDI-TOF mass spectrometry platform (Sequenom, Inc., San Diego, CA)[7]. In brief, polymerase chain (PCR) and extension reactions were designed using MassARRAY design software, and were carried out using 2.5 ng of template DNA. Unincorporated nucleotides in the PCR product were deactivated using shrimp alkaline phosphatase. Amplification of the SNP site was carried out using the MassExtend primer and involved the use of specific d/ddNTP termination mixes determined using MassARRAY assay design software. Primer extension products were then cleaned and spotted onto a SpectroChip. The chips were scanned using a mass spectrometry workstation (Bruker) and the resulting spectra were analyzed and genotypes determined using the Sequenom SpectroTYPER-RT software.

We genotyped PsA probands and controls for the following polymorphisms: *NFKB1*: in the promoter region, -94delATTG and the non-synonymous coding SNP rs4648065, rs4648072, rs4648085, rs4648086, and rs4648099; *RelA*: promoter SNP rs11568292, coding SNP rs7116571, and 3′ SNP rs2009453 and rs6591183; and *NFKBIA*: promoter SNP -410 (rs2233409), -642 (rs2233408), -673 (rs2233407), -949 (rs2233406), and 3′ SNP 2643 (rs8904), 2758 (rs696), and 3053 (rs2273650).

Statistical analysis. Chi-square analysis was used to test the single locus associations between SNP in NF-κB complex and PsA. Associations between multilocus haplotypes and case or control status were tested using the software PHASE, version 2.1, a haplotype reconstruction method that assesses similarity between haplotypes using arguments based in coalescent theory[8,9]. A permutation test examines the similarity of haplotype distributions between cases and controls. Since the algorithm considers haplotype similarity, the permutation test has power even when the number of haplotypes is large. To evaluate power of our study, given our sample size, simulation studies were done using the genotype relative risk (GRR) methods described by Risch and Merikangas[10]. If the high risk allele frequency (p) is greater than or equal to 0.1, the power to detect a GRR of 2.0 is 77%, and if (p) is greater than or equal to 0.3, the power to detect GRR of 1.75 is 85%.

RESULTS

Two hundred and thirty-four patients with PsA (52% male) and 88 ethnically matched controls (62% male) were studied. All were Caucasian of North European descent and considered native to Newfoundland. Mean age ± standard deviation of the patients with PsA was 50.0 ± 10.9 years; age at onset of psoriasis was 29.4 ± 14.3 years; and age at onset of PsA was 37.9 ± 11.3 years. All genotypes for the controls satisfied the Hardy-Weinberg equilibrium. Not all SNP were successfully genotyped in every individual.

With respect to single locus associations, none of the SNP tested were found to be associated with PsA in the Newfoundland population (Table 1). In particular, the allele frequency for the *NFKB1* -94delATTG was 41.7% in cases and 41.6% in controls (p = 0.97). For the 5 non-synonymous coding SNP of *NFKB1* (rs4648065, rs4648072, rs4648085, rs4648086, rs4648099), the genotypes were all homozygous for all cases and controls, with the exception of one control who was heterozygous for SNP rs4648072.

For the *RelA* 3′ SNP rs2009453, the minor allele (C) had a frequency of 42.2% compared to 40.3% in cases and controls, respectively, (p = 0.67). For the 3′ SNP rs6591183, the minor allele (A) frequency was 44.1% compared to 38.6% in cases and controls, respectively, (p = 0.23). The *RelA* promoter SNP rs11568292 and coding SNP rs7116571 were found to be homozygous in all patients and controls, except for one patient who was heterozygous for rs11568292.

The *NFKBIA* promoter SNP -410 (rs2233409) was found to have a minor allele (T) frequency of 25.0% in cases compared to 28.1% in controls (p = 0.45); for SNP -673

Table 1. Association of selected SNP in the *NFKB1, RelA*, and *NFKBIA* genes in PsA.

SNP	Genotype	PsA Patients with Each Genotype, n (%)	Controls with Each Genotype, n (%)
NFkB1-94delATTG	ATTG	75/224 (33.5)	26/83 (31.3)
	ATTG/DEL	111/224 (49.5)	45/83 (54.2)
	DEL/DEL	38/224 (17.0)	12/83 (14.5)
RelA rs2009453	CC	32/193 (16.6)	15/88 (17.0)
	CT	99/193 (51.3)	41/88 (46.6)
	TT	62/193 (32.1)	32/88 (36.4)
RelA rs6591183	AA	36/193 (18.6)	14/88 (15.9)
	AG	99/193 (51.0)	40/88 (45.5)
	GG	59/193 (30.4)	34/88 (38.6)
NFKBIA-410 (rs2233409)	CC	120/220 (54.5)	47/84 (56)
	CT	90/220 (41.0)	27/84 (32.1)
	TT	10/220 (4.5)	10/84 (11.9)
NFKBIA-673 (rs2233407)	AA	191/216 (88.4)	77/84 (91.7)
	AT	25/216 (11.6)	7/84 (8.3)
	TT	0/216 (0)	0/84 (0)
NFKBIA-949 (rs2233406)	CC	96/211 (45.5)	40/84 (47.6)
	CT	98/211 (46.4)	30/84 (35.7)
	TT	17/211 (8.1)	14/84 (16.7)
NFKBIA 2578 (rs696)	AA	20/222 (9.0)	8/88 (9.1)
	AG	86/222 (38.7)	37/88 (42.0)
	GG	116/222 (52.3)	43/88 (48.9)

(rs2233407), the minor allele (T) frequency was 5.8% versus 4.2%, respectively, (p = 0.42); for SNP -949 (rs2233406), the minor allele (T) frequency was 31.3% versus 34.5%, respectively, (p = 0.60); and for SNP 2578 (rs696), the minor allele (A) frequency was 28.4% versus 30.5% in cases and controls, respectively, (p = 0.21). For the remaining *NFKBIA* promoter SNP -642 (rs2233408) as well as the 3′ SNP 2643 (rs8904) and 3053 (rs2273650), the genotypes were all homozygous for all patients and controls.

Haplotypes were formed with 2 markers for *RelA* (rs2009453 and rs6591183) and 4 markers for *NFKBIA* [-410 (rs2233409), -673 (rs2233407), -949 (rs2233406) and (rs696)]. No associations were found for haplotypes for *RelA* (0.37) and *NFKBIA* (0.90). We re-analyzed all these markers as well as *NFKB1* -94delATTG for gene/gene interaction and again found no association (p = 0.82).

Results were further analyzed to determine any relationship between the minor allele frequency of each genotype, gender, and early onset of PsA (defined as onset of psoriasis prior to age 40 yrs). No such association was observed.

DISCUSSION

As noted in a recent editorial, NF-κB serves as a "master switch" for the inflammatory cascade in rheumatic disease as it is critically linked to many genes that result in synovitis such as proinflammatory cytokines and metalloproteinases[11]. The NFKB1 protein is found most often bound with RELA to form the NF-κB complex. The complex is inactive in the cytoplasm bound to an I-kappa-B inhibitory protein (NFKBIα) and is activated when the I-kappa-B kinases (IKBKA or IKBKB) phosphorylate serine residues on the NFKBIα protein, marking it for destruction via ubiquitination. The active NF-κB complex then is transported to the cell nucleus and binds DNA at κB-binding motifs, thereby activating gene expression[13]. Inappropriate activation of NF-κB has been linked to inflammatory events associated with autoimmune arthritis, asthma, septic shock, lung fibrosis, and atherosclerosis[14,15]. There are no previous studies that have examined the role of NF-κB in PsA.

We did not observe an association between PsA and SNP of components of the NF-κB complex (*NFKB1*, *RelA*, and *NFKBIA*). In particular we were unable to find an association between the *NFKB1* -94delATTG polymorphism and PsA. Our results strongly suggest that the major *NFKB1* -94delATTG variant, which has been associated with ulcerative colitis[5], does not play a role in PsA. However, we cannot rule out the possibility that an association exists for other SNP variants in genes in the NF-κB pathway or that there is an association in other populations, as the Newfoundland population may not be representative of other admixed Caucasian populations[16]. Furthermore as the majority of our cohort had either polyarticular disease (61.4%) or oligoarticular disease (33.6%), we cannot rule out the possibility that selected SNP may be associated with the other subtypes of PsA, which were quite small in our cohort. Finally, we also acknowledge that because of our sample size, we would have been unlikely to detect small differences in allele frequencies, as reflected in our *post hoc* power analysis.

Based on our observations, we conclude that there is no association between SNP of components of the NF-κB complex in the Newfoundland population. The possibility remains that novel SNP of these genes or genes further up or downstream in the NF-κB pathway may contribute to dysfunction of the inflammatory process leading to PsA.

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