# IL-23R Polymorphisms in Patients with Ankylosing Spondylitis in Korea

IL-HOON SUNG, TAE-HWAN KIM, SO-YOUNG BANG, TAE-JONG KIM, BITNARA LEE, LYNETTE PEDDLE, PROTON RAHMAN, CELIA M.T. GREENWOOD, PINGZHAO HU, and ROBERT D. INMAN

***ABSTRACT.*** ***Objective.*** IL23R polymorphisms have been shown to have a significant association with ankylosing spondylitis (AS). To date, these studies have been restricted to Caucasian patients with AS. Our study addresses this relationship in Korean patients with AS.

***Methods***. A total of 451 patients with AS and 392 ethnically matched healthy controls were enrolled. All patients were native Koreans with AS satisfying the modified New York criteria. In total, 10 single nucleotide polymorphisms (SNP) within the IL-23R gene cluster were genotyped.

***Results***. No IL-23R SNP were found to be associated with AS in Koreans.

***Conclusion***. The association of IL23R and AS that is seen in Caucasian patients with AS is not present in Korean patients with AS. (First Release April 15 2009; J Rheumatol 2009;36:1003–5; doi:10.3899/jrheum.081121)

*Key Indexing Terms:*
IL23R ANKYLOSING SPONDYLITIS

The hallmark of ankylosing spondylitis (AS) is acute and chronic inflammation in the sacroiliac joints, as well as sites of ligamentous and tendinous insertions into bone. Over time, chronic spinal inflammation can lead to complete spinal fusion, a process referred to as ankylosis, and often associated with progressive loss of spinal mobility. The pathogenesis of AS is unknown, but it is well established that genetic factors play a major role in susceptibility to AS. Although HLA-B27 is recognized to be the major gene associated with AS, a role for genes outside the HLA region is increasingly being recognized[1,2].

Interleukin 23 (IL-23), a key cytokine in innate and adaptive immune systems, stimulates a CD4+ helper T cell population producing IL-17. IL-23 is very important in animal models of autoimmune arthritis and inflammatory bowel disease (IBD)[3,4]. Synovial p40 IL12/23 levels are higher in patients with spondyloarthropathy (SpA) compared to osteoarthritis[5]. IL-23 receptor (IL-23R) is a potent proinflammatory cytokine, which is a key factor in the regulation of Th17 cells. IL-23R polymorphisms have recently been associated with SpA including IBD, psoriasis, and AS in Caucasian populations[6-11]. IL-23R may be of potential relevance in AS, as there is clinical, immunological, and genetic evidence suggesting an overlap between AS, psoriasis, and IBD. Recently, investigators have reported that the Arg381Gln single-nucleotide polymorphism (SNP) of the IL-23R gene, located on chromosome 1p31, confers a strong protective effect against AS in Caucasians[11]. However, there have been few studies on these SNP in Asian patients with AS[12], particularly Koreans. As previous studies have focused primarily on Caucasian populations of North European ancestry, we set out to study IL-23R variants in a relatively homogenous Korean population with AS.

## MATERIALS AND METHODS

*Patients and controls.* A total of 451 patients with AS and 392 ethnically matched, healthy controls were enrolled for our study. The AS population included 419 men and 32 women, age 35.8 ± 8.79 years [mean ± standard deviation (SD)]. All patients were native Koreans with AS satisfying the modified New York criteria[13]. Informed consent was obtained from all patients. Clinical information was collected systematically. Of these patients with AS, 3 (0.007%) had IBD and 4 (0.009%) had psoriasis. The control population included 325 men and 67 women, with age of 30.2 ± 6.72 years (mean ± SD). Healthy controls were screened by questionnaire to exclude those with a personal or familial history of arthritis. The study was approved by the ethics committee of Hanyang University in Korea.

*Genotyping of IL-23R SNP.* DNA was extracted using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA). In total, 10 SNP within the IL-23R gene cluster were genotyped in cases and controls. These SNP were selected on the basis of the findings of the recent IBD genome-wide scan and study of Caucasians with AS[6,11]. The 10 SNP were rs1004819, rs7517847, rs10489629, rs2201841, rs11465804, rs11209026,

*From Hanyang University College of Medicine, and the Hospital for Rheumatic Diseases, Seoul, Korea; Memorial University of Newfoundland, Department of Public Health Sciences, St. John's, Newfoundland; Hospital for Sick Children Research Institute, Program in Genetics and Genome Biology; Toronto Western Hospital and the University of Toronto, Toronto, Ontario, Canada.*

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*I-H. Sung, MD, PhD; T-H. Kim, MD, PhD; S-Y. Bang, MD; B. Lee, BSc, Hanyang University College of Medicine, and the Hospital for Rheumatic Diseases; T-J. Kim, MD, PhD, Chonnan National University Hospital; P. Rahman, MD, FRCPC; L. Peddle, BSc, Memorial University of Newfoundland, Department of Public Health Sciences; C.M.T. Greenwood, PhD, Associate Scientist; P. Hu, MSc, Hospital for Sick Children Research Institute, Program in Genetics and Genome Biology; R.D. Inman, MD, FRCPC, Toronto Western Hospital and the University of Toronto.*

*Address reprint requests to Dr. T-H. Kim, The Hospital for Rheumatic Diseases, Hanyang University, Seoul 133-792, South Korea. E-mail: thkim@hanyang.ac.kr*

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rs1343151, rs10889677, rs11209032, and rs1495965. Reactions were multiplexed where possible. The detection of SNP was performed using the chip-based matrix-assisted laser desorption ionization time-of-flight mass spectrometry platform (Sequenom, San Diego, CA, USA). Briefly, polymerase chain reaction (PCR) and extension reactions were designed using MassArray Design software (Sequenom). Primers were obtained from Integrated DNA Technologies (Coralville, IA, USA). The PCR primers were used to amplify 5 ng of genomic DNA using standard conditions for MassArray genotyping.

*Statistical analysis.* Tests of Hardy-Weinberg equilibrium (HWE) were performed for all polymorphic IL-23R SNP, which have HWE p values between 0.15 and 0.79 in control group and 0.07–1 in case group. Single-marker case-control differences were evaluated for all polymorphic IL-23R SNP, using chi-squared tests with 2 degrees of freedom (df), comparing the 3 genotypes in cases versus controls. Further, the Cochrane-Armitage test for trend[14] across the 3 genotypes (1 df), and genotypic chi-squared tests were performed for association between each marker and trait using the qtscore function in the GenABEL R package[15]. All SNP as shown in Table 1 met the quality control (QC) requirement (minor allele frequence > 0.01). Therefore, all 8 SNP met the QC requirements (made in genome-wide association analysis), such as SNP call rate > 95%, HWE p value > $1 \times 10^{-6}$ (0.000001) and minor allele frequence > 0.01. More than 99% of samples also met the QC requirements, such as sample call rate > 95%. In haplotype-based association analysis, the algorithm proposed by Schaid, *et al*[16] was used to test the association between haplotypes and the trait. The algorithm was implemented in the GenABEL R package (scan.haplo and scan.haplo. 2D functions). We also tested the association between haplotypes constructed based on 3 consecutive loci with the trait.

## RESULTS

In total, 843 subjects were genotyped (451 patients with AS, 392 controls) for IL-23R polymorphisms. Of patients with AS, 98.2% were HLA-B27-positive. In total, 10 SNP within the IL-23R were genotyped, but 2 (rs11456804, rs11209026) were not polymorphic in this population and were removed from analysis. No SNP was found to be associated with AS (Table 1). Of the patients with AS, 153 (34%) had uveitis and 227 (50.3%) had peripheral arthritis. No SNP was found to be associated with AS patients with uveitis or peripheral arthritis. Further, haplotype analyses including either 2 or 3 adjacent markers did not reveal any significant associations.

## DISCUSSION

Spondyloarthropathy (SpA) refers to a family of arthritides of unknown etiology with both peripheral and axial manifestations sharing clinical and radiological features as well as genetic predisposing factors. SpA includes AS, reactive arthritis, psoriatic arthritis (PsA), and arthritis related to IBD, as well as undifferentiated SpA. It is evident that IL-23R polymorphisms are associated with multiple disease states among SpA. In AS, there was an association with the Caucasian British, Spanish, Canadian, and American cohorts[9-11,17]. Further, association has been noted in Crohn's disease[6,7,18], psoriasis, and PsA[9]. An association with a coding SNP rs11209026 and an intergenic SNP 11465804 appears to be prominent in all these studies. However, these 2 SNP are not polymorphic in the Korean population. When the analysis was repeated excluding AS patients with psoriasis and IBD, no SNP was found to be associated with AS. It is possible that an association might be observed with larger numbers of subjects. Some genes over and above HLA-B27, besides IL-23R, have been recognized as genetic markers in SpA. CARD15 is known to be associated with IBD. The IL-1 gene cluster has been implicated in Caucasian patients with PsA and AS[19]. However, these genes are not associated in Koreans with AS[20-22]. These findings suggest that population stratification should really be taken into account in genetic studies, and highlight the importance of investigating additional racial backgrounds other than Caucasians.

Ours is the first study of this candidate gene in non-Caucasians with AS, and the findings differ significantly from recent studies in Caucasian populations, despite the common clinical features between Korean and Caucasian patients with AS and their shared association with HLA-B27. In addition to raising hypotheses about differing genetic pathways to common clinical outcomes, our study is a reminder of the critical issue of appropriate matching of cases and controls in genetic association studies in AS, which are actively being pursued in large population cohorts.

*Table 1.* Allele frequency and odds ratios of IL23R variants in a Korean cohort* with ankylosing spondylitis.

| SNP rs no. | Allele | | Minor Allele Frequency | | |
|---|---|---|---|---|---|
| | 1 | 2 | Cases, n = 451 | Controls, n = 392 | OR (95% CI) |
| 1004819 | T | C | 0.414 | 0.419 | 1.03 (0.81–1.31) |
| 7517487 | T | G | 0.391 | 0.414 | 1.11 (0.88–1.41) |
| 10489629 | A | G | 0.263 | 0.255 | 1.03 (0.80–1.33) |
| 2201841 | C | T | 0.267 | 0.257 | 1.04 (0.81–1.34) |
| 11456804 | T | G | — | — | 0.91 (0.57–1.45) |
| 11209026 | G | A | — | — | 1.03 (0.80–1.33) |
| 1343151 | C | T | 0.043 | 0.048 | 0.97 (0.76–1.22) |
| 10889677 | A | C | 0.268 | 0.260 | 1.01 (0.80–1.28) |
| 11209032 | G | A | 0.497 | 0.489 | 1.03 (0.81–1.31) |
| 1495965 | G | A | 0.492 | 0.494 | 1.11 (0.88–1.41) |

* Study population was 854 subjects (451 cases and 391 controls) and 8 SNP with 13 missing values. Two SNP (rs11456804, rs11209026) were not polymorphic. SNP: single-nucleotide polymorphism.

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