Analysis of CARD15 Polymorphisms in Korean Patients with Ankylosing Spondylitis Reveals Absence of Common Variants Seen in Western Populations

TAE-HWAN KIM, PROTON RAHMAN, JAE-BUM JUN, HYE-SOON LEE, YONG-WOOK PARK, HO JOON IM, TARA SNELGROVE, LYNETTE PEDDLE, DAVID HALLETT, and ROBERT D. INMAN

ABSTRACT. ***Objective.*** Substantial epidemiological and genetic evidence suggests that ankylosing spondylitis (AS) is likely due to an interplay of genetic and environmental factors. Recently, *CARD15*, located in chromosome 16q12, has been established as a disease susceptibility gene for Crohn's disease, Blau syndrome, and possibly psoriatic arthritis. Association studies in admixed populations from Northern European ancestry noted no such association between *CARD15* mutations and AS. However, a homogenous population has yet to be studied. We investigated the prevalence of the 3 common *CARD15* variants in a homogenous Korean population with AS.

Methods. All subjects were native Koreans with AS satisfying the modified New York criteria. Korean controls were examined and confirmed to be unaffected by AS. Subjects with AS were genotyped for the R702W, G908R, and Leu1007fsinsC variants of *CARD15* using mass array MALDI-TOF mass spectrometry.

Results. A total of 205 AS subjects and 200 controls were genotyped. No subject with AS had any variants at the 702 and 1007 sites of *CARD15*. Only one subject was heterozygous for the 908 variant. The overall genotype frequency in AS for any *CARD15* variant was 0.5%. No control had any of the 3 *CARD15* variants.

Conclusion. Our findings indicate that the *CARD15* gene is not a major contributor to AS susceptibility in the Korean population. (J Rheumatol 2004;31:1959–61)

Key Indexing Terms:
CARD15 ANKYLOSING SPONDYLITIS

Ankylosing spondylitis (AS) is an inflammatory rheumatic disease characterized by pain and stiffness in the spine and sacroiliac joints. Substantive epidemiological evidence suggests that AS is likely due to an interplay of genetic and environmental factors. With respect to the genetic factors in AS, a major role for genes outside the HLA region is increasingly being recognized[1]. Recently, the *CARD15* gene located in chromosome 16q12 has been established as a susceptibility gene for Crohn's disease (CD)[2], Blau syndrome[3], and possibly psoriatic arthritis[4]. The major phenotype from all 3 of these *CARD15* associated diseases (inflammatory colitis, psoriasis, and uveitis) often co-segregates in subjects with AS. For instance, AS frequently coexists with inflammatory bowel disease, and subclinical bowel inflammation is found in a majority of patients with AS[5]. Further, AS and CD also share similar pathways of inflammation and both present circumstantial evidence implicating infectious triggers. In a genome-wide scan of 185 families containing 255 affected sibling pairs, the strongest non-MHC linkage was noted on chromosome 16q[1]. This is a pleiotropic autoimmune region that overlaps *CARD15*, as multiple autoimmune diseases have mapped to this region. However, 4 association studies in admixed populations from Northern European Ancestry have noted no association between selected variants of *CARD15* and AS[6-9]. Interestingly, however, disease activity, as measured by the Bath Ankylosing Spondylitis Activity Index (BASDAI), has been associated with the P268S variant of *CARD15* in patients with AS associated with inflammatory bowel disease[10]. As the studies to date have focused on relatively admixed populations of North European ancestry, we set out to determine the prevalence of the 3 common *CARD15* variants in a homogenous Korean population with AS.

MATERIALS AND METHODS

Patients. Patients with AS were drawn from the Spondylitis Clinic at the

From the Hospital for Rheumatic Diseases, Hanyang University, Seoul, Korea; Toronto Western Hospital, University of Toronto, Toronto, Ontario, Canada; Memorial University of Newfoundland, St. John's, Newfoundland, Canada; Gachon Medical School, Gil Medical Center, Inchon, Korea; and Mount Sinai Hospital, Toronto Canada.

T-H. Kim, MD, PhD, Assistant Professor, Hanyang University; P. Rahman, MD, Professor, Memorial University; J-B. Jun, MD, PhD, Associate Professor; H-S. Lee, MD, PhD, Assistant Professor; Y-W. Park, MD, PhD, Clinical Fellow, Hanyang University; H.J. Im, MD, PhD, Assistant Professor, Gachon University; T. Snelgrove, BSc, Research Associate; L. Peddle, MSc, Research Associate, Memorial University; D. Hallett, MSc, Biostatistician, Critical Care Unit, Mount Sinai Hospital; R.D. Inman MD, Professor, 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

Submitted January 15, 2004; revision accepted May 17, 2004.

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Hospital for Rheumatic Diseases, Hanyang University. All AS subjects satisfied the New York criteria[11]. The controls were from the same ethnic background and were documented to be unaffected by AS. The study was approved by the regional ethics committee, and written informed consent was obtained from all participants. The Korean population in Seoul is genetically homogenous, with very little ethnic or racial heterogeneity.

Genotyping. Subjects with AS were genotyped for the following 3 *CARD15* variants: (1) R702W (SNP8, exon 4), (2) G908R (SNP12, exon 8), and (3) 1007fs (SNP13, exon 11). These 3 *CARD15* variants were selected for study as they are consistently shown to be associated with CD, each exerts an independent effect, and they collectively account for over 80% of the recognized variants in the North European Caucasian population. For determination of the *CARD15* (Genbank accession number 64127; MIM 605956) variants, 2.5 ng of genomic DNA was used for each polymerase chain reaction (PCR) procedure. For each variant, a standard PCR protocol was performed using primers listed in Table 1 to amplify a 99–111 bp fragment containing the polymorphic site of interest. After template amplification, all unincorporated dNTP were deactivated and the primer extension reaction was prepared. This reaction involves using the corresponding mass extend primer and a d/ddNTP mix specific to each reaction. The samples were then loaded onto a 384 DNA chip and scanned using the MALDI-TOF mass spectrometer.

Statistical methods. Allele and genotype frequencies were compared in the cases and controls using Fisher's exact test.

RESULTS

In total 405 subjects were genotyped (205 AS patients and 200 controls). Ninety-two percent of the AS patients were male (n = 188) and 8% (n = 17) were female. The mean age of the AS patients at study entry was 31.4 years (± 9.8) and mean disease duration was 11.6 years (± 6.6).

Strikingly, only one patient of the entire cohort of 405 patients carried a *CARD15* variant. Specifically, none of the AS subjects were carriers for R702W or 1007fs variants of the *CARD15* gene. With respect to the G908R variant, one AS subject was heterozygous for this variant. Thus the carrier frequency for any of the *CARD15* variants was 0.5%. For the controls, none of the 200 subjects had any of the 3 *CARD15* variants. Thus in our study, none of the common variants of the *CARD15* gene (R702W, G908R, and 1007fs) were present in the Korean control population. Second, there was no association between these *CARD15* variants and AS in the Korean population (p = 1.0).

DISCUSSION

The usual carrier frequency for Western controls for the 3 recognized *CARD15* mutations varies between 10% and 20%[12]. Our study revealed a conspicuous absence of these variants in the Korean population. This finding bears a striking resemblance to 2 recent studies that examined the prevalence of *CARD15* variants in Japanese subjects with CD and corresponding Japanese controls. In the first study, none of the 350 Japanese CD subjects nor 292 controls carried any *CARD15* variant[13]. In the second study, only CD subjects were genotyped, and only one of the 483 subjects carried a *CARD15* mutation[14]. In striking contrast, 30%–40% of CD subjects from Western populations carry a *CARD15* mutation[12]. Together with these Japanese reports, our study highlights the importance of taking ethnic differences into account when assessing the role of candidate genes in complex diseases. Asian populations (Japanese and Korean) are clearly very distinct genetically from Western populations. This study demonstrates foremost that appropriate selection of controls from the major ethnic groups must always remain the goal in these case-control studies. Second, population stratification should be considered as a confounding variable in genetic association studies, especially when the comparability of ethnicity of the cases and controls is uncertain.

Finally, we are able to conclude that the common *CARD15* variants do not play a major role in AS in the Korean population. It is conceivable that other mutations in the *CARD15* gene are associated with AS in this population. However, when the entire 12 exons of *CARD15,* including its 5' region, were directly sequenced in the Japanese CD cohort, only 2 rare variants were discovered, and neither of these had an allele frequency greater than 5%[15]. This is in contrast with the Western population, in which 67 variants have been noted, including 9 variants with an allele frequency greater than 5%[16]. Thus, further mutational analysis of the *CARD15* gene in a Korean population is not likely to be rewarding. On the other hand, the interaction of genetic and environmental factors in the pathogenesis of AS, itself a complex relationship,

Table 1. Primers labeled –1 and –2 are forward and reverse primers that amplify a region of DNA containing the SNP site. Primers labeled –ME were used in a primer extension reaction and hybridize directly adjacent to the SNP site. Primer sequences were synthesized by Integrated DNA Technologies, Coralville, IA, USA.

Variant Site	Primer	Primer Sequence	Product Size	d/ddNTP Mix
702	702–1	5′- ACG TTG GAT GAT GGA GTG GAA GTG CTT GCG -3′	111 bp	
	702–2	5′- ACG TTG GAT GAG TGC CAG ACA TCT GAG AAG -3′		
	702–ME	5′- CAT CTG AGA AGG CCC TGC TC -3′		ACG
908	908–1	5′- AGC GGA TAA CGT CTG TTG ACT CTT TTG GCC -3′	110 bp	
	908–2	5′- AGC GGA TAA CTG ATC ACC CAA GGC TTC AGC -3′		
	908–ME	5′- TCG TCA CCC ACT CTG TTG C -3′		ACT
1007	1007–1	5′- AGC GGA TAA CAA CTG CAT CAC CTA CCT AGG -3′	99 bp	
	1007–2	5′- AGC GGA TAA CCT TAC CAG ACT TCC AGG ATG -3′		
	1007–ME	5′- ATG GTG TCA TTC CTT TCA AGG G -3′		ACT

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must further consider that the same clinical phenotype can be seen in remarkably diverse genetic and environmental backgrounds.

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