Genetic Studies of Ankylosing Spondylitis in Koreans Confirm Associations with *ERAP1* and 2p15 Reported in White Patients

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ABSTRACT. ***Objective.*** Investigators from the Australo-Anglo-American Spondyloarthritis Consortium have reported additional genes associated with ankylosing spondylitis (AS) susceptibility including *IL1R2*, *ANTXR2*, and gene deserts at 2p15 and 21q22. We evaluated these new candidate genes in a large cohort of Korean patients with AS.

Methods. A group of 1164 patients with AS and 752 healthy controls were enrolled for our study. Eight single-nucleotide polymorphisms (SNP) were analyzed to define genetic association with AS by MassARRAY system.

Results. Significant positive associations of AS with endoplasmic reticulum aminopeptidase 1 SNP, rs27037 ($p = 1.31 \times 10^{-4}$), and rs27434 ($p = 4.59 \times 10^{-6}$), were observed. The rs10865331 of gene desert at 2p15 also showed a significant association with AS ($p = 4.63 \times 10^{-5}$).

Conclusion. This is the first confirmation in a nonwhite population that genetic polymorphisms of rs27037, rs27434, and rs10865331 are associated with AS, implicating common pathogenetic mechanisms in Korean and white patients with AS. (J Rheumatol First Release Nov 1 2010; doi:10.3899/jrheum.100652)

Key Indexing Terms:
ANKYLOSING SPONDYLITIS *ERAP1* 2p15 KOREA
SINGLE-NUCLEOTIDE POLYMORPHISM

Ankylosing spondylitis (AS) is a chronic inflammatory disorder primarily involving the sacroiliac joints and spine. It usually affects young people, and multiple genetic factors are implicated in conferring susceptibility to AS. *HLA-B27* remains the major susceptibility gene, and is present in 80%–95% of AS cases. Several theories to explain the *HLA-B27* association have been proposed, but the pathogenesis and mechanisms of its association with AS are not well defined[1,2,3].

In 2007, an association study was published, reporting findings of 14,500 nonsynonymous single-nucleotide polymorphisms (SNP) in 1500 white controls and 1000 white patients with AS[4]. As expected, MHC genes showed the strongest association with AS, but 2 non-MHC genes, endoplasmic reticulum aminopeptidase 1 (*ERAP1*) and interleukin 23 receptor (*IL-23R*) also showed strong association. Recently, the Australo-Anglo-American Spondyloarthritis Consortium (TASC) reported that additional genes are implicated in AS susceptibility, including *IL1R2*, *ANTXR2*, and gene deserts at 2p15 and 21q22[5]. It has now been agreed that 5 genes/genetic regions are definitively associated with AS (MHC, *IL23R*, *ERAP1*, 2p15, and 21q22)[6]. But these studies were performed exclusively in cohorts of white European descent, and only limited information has been reported from Asian countries[7,8].

We have reported that *ERAP1* is associated with AS in Koreans, while *IL-23R* is not[8,9]. In our current study, we evaluate the new candidate genes in a large cohort of Koreans with AS and healthy controls.

MATERIALS AND METHODS

Subjects. A total of 1164 patients with AS and 752 ethnically matched, healthy controls were enrolled for this study. All cases and controls were native Koreans, and AS cases satisfied the modified New York criteria[10]. Clinical information was collected systematically and informed consent was obtained from all participants. The ethics committee of Hanyang University in Korea approved our study.

Healthy controls were screened by questionnaire to exclude those with a personal or familial history of arthritis or spondyloarthropathy. Eighty-eight percent of the AS cases were male, age of symptom onset was

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Supported by Hanyang University, Korea, in program year 2010 (HY-2010-I).

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Accepted for publication September 1, 2010.

22.8 ± 8.9 years (mean ± SD), and mean age at study entry was 35.3 ± 9.8 years. Of these AS cases, 96.5% were *HLA-B27*-positive (n = 1078/1117), 21.4% (243/1140) had juvenile-onset AS (age at symptom onset < 16 yrs), 24.7% (n = 278/1128) had uveitis, and 41.7% (n = 465/1116) had peripheral arthritis. The control population included 658 men (87.6%) with age of 31.4 ± 7.3 years.

Methods. Peripheral blood from cases and controls was collected in EDTA tubes, and genomic DNA was isolated using a Puregene blood DNA kit (Qiagen). A total of 1916 Korean samples were genotyped for 8 SNP. The MassARRAY® system (Sequenom, San Diego, CA, USA) was used to genotype each study participant in a 2-well reaction using Assay Designer 3.1; SNP 1–7 (rs10865331, rs3734523, rs4672495, rs2310173, rs2242944, rs27434, and rs4333130) were run as a multiplex reaction, and SNP 8 (rs27037) was run as a separate uniplex reaction. The reactions were processed following standard protocols for iPLEX™ chemistry, with the following exceptions: the concentration of shrimp alkaline phosphatase in the cleanup reaction was increased by 25%, and incubation at 37°C was increased by 15 min. Upon completion of the extension reaction, the products were cleaned, spotted onto SpectroChip II™ arrays, and scanned using a MassARRAY Compact Analyzer (Sequenom). Genotypes were determined using MassARRAY Typer v.4.0 software.

Statistical analysis. The frequencies and OR of the alleles and genotypes of all polymorphic SNP were calculated with a 95% CI, and a chi-squared test was used to compare the results between AS subjects and controls. Bonferroni correction was applied for multiple comparisons. The observed allelic frequencies for these SNP satisfied the Hardy-Weinberg equilibrium in controls.

RESULTS

In total, 1916 subjects (1164 patients with AS and 752 controls) were genotyped. Among the 8 SNP, rs3734523 and rs4333130 SNP were rare in the Korean population (Table 1). Therefore, these SNP were excluded from analysis. Significant positive association of AS with 2 SNP of *ERAP1*, rs27037 ($p = 1.3 \times 10^{-4}$) and rs27434 ($p = 4.6 \times 10^{-6}$), were observed. SNP rs10865331 at chromosome 2p15 was also found to be significantly associated with AS ($p = 4.6 \times 10^{-5}$; Table 2).

When we analyzed the clinical symptoms among the AS cases, the rs27434 (OR 1.4, 95% CI 1.1–1.7, $p = 1.5 \times 10^{-3}$) was significantly associated with juvenile-onset AS compared with adult-onset AS. There were no significant differences between AS cases with peripheral arthritis and those without peripheral arthritis, nor with AS cases with and without uveitis (Table 3).

DISCUSSION

We present 2 principal findings concerning the genetics of Korean AS in our case-control study. First, significant positive associations of AS with *ERAP1* SNP rs27037 and rs27434 were observed. Second, we demonstrated that rs10865331 at the chromosome 2p15 intergenic region was significantly associated with AS in Koreans. With completion of the Human Genome Project and the International HapMap Projects, genome-wide association studies (GWAS) are offering an efficient and effective method of discovering genetic associations with human diseases. Hundreds of loci for over 40 diseases have already been identified, producing new associations as well as confirming previous ones[11]. GWAS have ushered in major advances in AS genetics as well. In addition to confirming the well established MHC association with AS, at least 6 other loci have been found to be associated with AS. Many of these findings were seen in studies of white patients of European ancestry who had AS.

We have confirmed that certain SNP of *ERAP1* are associated with AS in Koreans, while other genes including *IL-1*, *CARD15*, *TLR-4*, and *IL-23R* are not associated with AS in this population[8,9,12,13]. *ERAP1* SNP rs27044 and rs30187 showed significant association with AS, but SNP rs17482078, rs10050860, and rs2287987 showed no association with AS in Korea[8]. Davidson, *et al* reported that in the Chinese Han population, *ERAP1* polymorphisms were associated with AS, but SNP across *IL23R* were not[7]. When 38 SNP of *ERAP1* were analyzed, rs27037 showed significant association with AS (p = 0.012), but 27434 did not (p = 0.14). In our study, these 2 SNP of *ERAP1* were significantly associated with AS, and some additional SNP of *ERAP1* also demonstrated strong association with AS among Asians. *ERAP1* is involved in trimming of peptides in the endoplasmic reticulum prior to HLA Class I presentation.

Table 1. Genotype and allele frequency of 8 single-nucleotide polymorphisms (SNP) in a Korean AS cohort. A total of 1916 subjects were genotyped (1164 cases, 752 controls) for 8 SNP with missing values. One SNP (rs3734523) was rare, and the rs4333130 SNP was not common.

Chromosomal Band	SNP	Gene	Allele		Cases, n = 1164 Genotypes			Total	MAF	Controls, n = 752 Genotypes			Total	MAF
			1	2	11	12	22			11	12	22		
2p15	rs4672495	—	T	G	939	197	12	1148	0.096	563	155	11	729	0.121
2p15	rs10865331	—	G	A	410	539	200	1149	0.409	318	328	87	733	0.342
2q11.2	rs2310173	*IL1R2*	G	T	505	515	125	1145	0.334	353	301	72	726	0.306
4q21.21	rs4333130	*ANTXR2*	T	C	1039	113	2	1154	0.051	656	77	5	738	0.059
5q15	rs27037	*ERAP1*	G	T	403	578	142	1123	0.384	343	280	89	712	0.322
5q15	rs27434	*ERAP1*	G	A	198	626	295	1119	0.543	216	336	167	719	0.466
6p22.2	rs3734523	—	C	T	1144	13	0	1157	0.006	725	13	1	739	0.010
21q22.2	rs2242944	—	G	A	346	575	230	1151	0.450	219	336	171	726	0.467

MAF: minor allele frequency; AS: ankylosing spondylitis.

Table 2. Odds ratios of 6 single-nucleotide polymorphisms (SNP) in a Korean ankylosing spondylitis cohort. OR and 95% CI were calculated by chi-squared test.

Chromosomal Band	SNP	Gene	Allele	OR (95% CI)	p
2p15	rs4672495	—	G	0.77 (0.63–0.95)	0.01
2p15	rs10865331	—	A	1.33 (1.16–1.52)	4.63×10^{-5}
2q11.2	rs2310173	*IL1R2*	T	1.14 (0.99–1.31)	0.08
5q15	rs27037	*ERAP1*	T	1.31 (1.14–1.51)	1.31×10^{-4}
5q15	rs27434	*ERAP1*	A	1.36 (1.19–1.56)	4.59×10^{-6}
21q22.2	rs2242944	—	A	0.93 (0.82–1.06)	0.30

Table 3. Allele frequency and susceptibility of 6 single-nucleotide polymorphisms (SNP) among patients with ankylosing spondylitis. Odds ratios and p values were obtained by chi-squared test.

			Uveitis, n = 278			Peripheral Arthritis, n = 465			JAS, n = 243		
Chromosomal Band	SNP	Gene	MAF	OR (95% CI)	p	MAF	OR (95% CI)	p	MAF	OR (95% CI)	p
2p15	rs4672495	—	0.089	0.88 (0.63–1.24)	0.47	0.096	1.01 (0.75–1.34)	0.97	0.081	0.79 (0.55–1.14)	0.20
2p15	rs10865331	—	0.416	1.05 (0.86–1.27)	0.64	0.395	0.91 (0.76–1.08)	0.27	0.407	0.98 (0.80–1.21)	0.87
2q11.2	rs2310173	*IL1R2*	0.322	0.94 (0.76–1.15)	0.53	0.343	1.08 (0.90–1.29)	0.42	0.322	0.93 (0.75–1.15)	0.49
5q15	rs27037	*ERAP1*	0.399	1.10 (0.90–1.34)	0.35	0.394	1.08 (0.91–1.29)	0.39	0.408	1.15 (0.94–1.42)	0.18
5q15	rs27434	*ERAP1*	0.564	1.12 (0.92–1.36)	0.27	0.551	1.04 (0.88–1.24)	0.64	0.606	1.40 (1.14–1.72)	1.46×10^{-3}
21q22.2	rs2242944	—	0.451	1.01 (0.84–1.23)	0.90	0.463	1.12 (0.94–1.32)	0.21	0.458	1.04 (0.85–1.28)	0.67

JAS: juvenile ankylosing spondylitis; MAF: minor allele frequency.

ERAP1 also binds directly to the extracellular domain of tumor necrosis factor receptor (TNFR)1 *in vitro* and promotes its interleukin 1ß-mediated ectodomain cleavage to generate TNFR1. But functional analysis of the polymorphic forms of ERAP has not been determined yet[6,14]. *ERAP1* is not associated with inflammatory bowel diseases that have clinical links to the spectrum of spondyloarthropathies such as AS[4]. Further studies of *ERAP1* in AS among Asians will be valuable because the prevalence of inflammatory bowel disease in Korean AS is very low.

The TASC GWAS demonstrated strong association of an intergenic region on chromosome 2p15 with AS[5]. No genes are known to be encoded at this locus, but TASC identified tags from a single long noncoding RNA transcript in AS cases and controls originating from this narrow locus (23 kb)[5]. Further research is required to fully characterize the noncoding RNA involved.

This is the first report on AS among Asians to demonstrate association with the 2p15 gene desert, which has been significantly associated with AS cases among whites. Except for *ERAP1* and 2p15, there were no associations with AS in the Korean population.

ACKNOWLEDGMENT

We are grateful to Matt A. Brown for assistance with drafting the manuscript.

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