ERAP1 Is Associated with Ankylosing Spondylitis in Han Chinese

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ABSTRACT. ***Objective.*** Genetic components play important roles in the incidence and development of ankylosing spondylitis (AS). Aminopeptidase regulator of tumor necrosis factor receptor shedding 1 (*ERAP1*) was recently found to be associated with AS in North American and British cohorts. We evaluated whether *ERAP1* is associated with AS in a Chinese Han population.

Methods. A sample of 50 patients and 50 healthy controls was recruited for preliminary screening for informative single-nucleotide polymorphisms (SNP). Then 6 SNP of suggestive significance in the initial screening were followed up in a large sample of 471 patients with AS and 456 ethnically matched controls. Diagnosis of AS followed the 1984 modified New York criteria. Linkage disequilibrium coefficient (D' and r^2) and haplotypes were estimated by Haploview.

Result. Two SNP (rs27434, p = 0.00039, and rs27529, p = 0.0083) in *ERAP1* other than that reported previously were found to be significantly associated with AS. Haplotype analysis using 5 SNP within 1 linkage disequilibrium block identified 2 risk haplotypes (GATGT and GACGT) and 1 protective haplotype (GGTGT) for AS.

Conclusion. Our study demonstrated that 2 novel SNP in *ERAP1* were associated with AS in the Han Chinese population, suggesting that *ERAP1* might confer genetic risk for AS in Han Chinese through the common mechanism shared by different populations, although the AS-associated SNP in *ERAP1* might be population-specific. (First Release Nov 15 2010; J Rheumatol 2011;38:317–21; doi:10.3899/jrheum.100013)

Key Indexing Terms:
ERAP1 ANKYLOSING SPONDYLITIS CHINA

Ankylosing spondylitis (AS) is a progressive chronic disease characterized by inflammatory low back pain, sometimes accompanied by peripheral arthritis, enthesis, iritis, spinal deformity, and ankylosis. The prevalence of AS ranges from 0.1% to 1.8% in Europe and is 0.24% in China[1,2]. Although genetic components have been proposed as important contributors to the pathogenesis of AS[3], the responsible genetic molecules and related mechanisms remain unclear.

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Supported by grants (2005A30801005) to J. Gu from the Foundation of Guangdong Province of China, clinical subject (2007) of the Ministry of Public Health of China and 5010 Subject (2009-2010) of Sun Yat-sen University.

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Accepted for publication September 17, 2010.

HLA-B27 is considered to be associated with the familial aggregation of AS[4], but it was estimated that it contributed to no more than 50% of the overall genetic risk for AS[5,6]. A recent genome-wide scan using 14,500 customized nonsynonymous single-nucleotide polymorphisms (SNP) revealed that *ERAP1* and *IL23R* were associated with AS in a group of Europeans, and specifically, the associations at rs27044, rs17482078, rs10050860, rs30187, and rs2287987 in *ERAP1* were reported[7]. Most recently, the association of *ERAP1* with AS was validated in a Korean group[8]. *ERAP1* encodes an endoplasmic reticulum-associated aminopeptidase (also known as ARTS1). This protein facilitates HLA class I presentation by processing peptides to optimal length[9,10]. It also binds and cleaves several cytokine receptors including interleukin 6 receptor (IL-6R), tumor necrosis factor R1 (TNFR1), and IL-1RII from cell surface and thus promotes receptor shedding[11,12,13,14].

We carried out a case-control association study to determine whether *ERAP1* is also associated with the incidence of AS in a Chinese population and whether it is correlated with clinical features.

MATERIALS AND METHODS

Subjects. All participants signed informed consent for their blood samples to be taken and used in this study, which was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University. Fifty patients and 50 healthy controls were recruited for preliminary screening

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for informative SNP. The sample for validation consisted of 471 patients with AS and 456 ethnically matched controls. Diagnosis of AS followed the 1984 modified New York criteria[15], and patients were assessed by at least 2 qualified rheumatologists. The clinical data included age, sex, family history, onset age, dactylitis, peripheral arthritis, hip joint involvement, iritis, enthesitis, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

Genotyping. Genomic DNA was obtained from peripheral blood samples using the phenol/chloroform extraction method[16]. To identify the candidate SNP in the promoter region (3000 base pairs upstream of the first exon), exonic region, and exon-intron junctions of the *ERAP1* gene, the fragments were amplified, respectively, using polymerase chain reaction (PCR) with specific primers (Table 1), and then sequenced with an ABI 3700 automated sequencer after purification of PCR products. In the validation stage, PCR-restriction fragment–length polymorphism (RFLP) was carried out to genotype 6 SNP. Briefly, each fragment flanking the SNP was PCR-amplified and then digested by the nucleases. Primers and nucleases designed for the PCR-RFLP analysis are shown in Table 1.

Statistical analysis. Hardy-Weinberg equilibrium by chi-squared test was used to evaluate the genotyping accuracy of each SNP. In the initial stage, allelic associations between cases and controls were performed using Fisher's exact test to generate significance, regarding the matching of sex and age between cases and controls. The magnitude of association was expressed as OR for each addition of 1 minor allele, with a 95% CI. In the followup stage, since age and sex were not matched between cases and controls (both $p < 0.0001$), multivariate logistic regression analysis was also performed using SAS 8.0 to test the correlations between the SNP and AS, adjusting for age and sex. To adjust for multiple testing on the 6 candidate SNP, p values were corrected by Bonferroni method[17]. Linkage disequilibrium (LD) coefficient (D' and r^2) and haplotypes were estimated by Haploview 4.1[18]. Population-attributable risk (PAR) was calculated as follows: PAR% = (It – I0)/It × 100%, where It is the incidence in the general population and I0 is the incidence in a nonexposed group.

Finally, relationships among a list of clinical measurements (age, sex, family history, onset site, dactylitis, peripheral arthritis, hip joint involvement, iritis, enthesitis, ESR, and CRP) and SNP were evaluated by SAS 8.0 and multivariate logistic regression with age and sex as covariates.

RESULTS

Clinical data. In the initial screening sample of 50 patients with AS and 50 controls, for patients, the mean age was 28.2 ± 6.7 years and the mean disease duration was 8.1 ± 7.1 years; the male to female ratio was 5.25. Most patients with AS (92%) were HLA-B27-positive, of which 20% had a family history of the disease. For controls, the mean age was 27.2 ± 7.1 years; male to female ratio was 5. Age and sex were not significantly different between AS cases and controls. Among those cases selected for validation, the mean age and mean disease duration were 28.7 ± 8.7 years and 7.07 ± 7.78 years, respectively. The male to female ratio was 5.73. HLA-B27 positivity was found in 86.7% (n = 408) of patients with AS, of which 21% had a family history of the disease. Among controls selected for validation, the mean age was 26.08 ± 8.88 years; male to female ratio was 1.65.

Initial screening for suggestive significant SNP. By using PCR-based direct sequencing, a total of 41 SNP were found in the promoter and exon regions of *ERAP1*. After stringent quality control filtering including deviation from Hardy-Weinberg disequilibrium, minor allele frequency, and homogeneity, 26 SNP were retained for analysis. The SNP that were associated with AS[7,8] (rs27044, rs17482078, rs10050860, rs30187, and rs2287987) were not included. Six SNP (rs26653, rs27434, rs27640, rs27529, rs26510, and rs27582) showed significant association ($p < 0.05$) in the initial sample, while the others did not, with p values ranging from 0.087 to 1.00.

Validation of SNP in a larger population. Six SNP (rs26653, rs27434, rs27640, rs27529, rs26510, and rs27582) that showed significance ($p < 0.05$) in the initial screening stage were subjected for validation in a sample of 471 AS cases and 456 healthy controls using PCR-RFLP assays. All 6 SNP were in Hardy-Weinberg equilibrium in the control group.

Single-marker association analysis for SNP in *ERAP1* (Table 2) showed that 2 SNP, rs27434 and rs27529, were significantly associated with AS. The frequency of risk allele A of rs27434 is higher in cases of AS (51%) than in controls (43%) (OR 1.38, 95% CI 1.09 ~ 1.74, $p = 0.00039$; after Bonferroni correction, $p_c = 0.002$), by adjustment for age and sex. The allele A of SNP rs27434 contributed to 7.4% of the overall genetic risk in AS. The frequency of risk allele G of rs27529 was higher in cases (60%) than in con-

Table 1. PCR-RFLP information for the 6 candidate SNP selected by sequencing.

SNP Detected	Type	Primer Sequence	Product Size, bp	RFLP Enzyme
rs26653	Nonsynonymous	F: 5' ACT CTG TGA CCG TGT AGT GA3' R: 5' AAC AGC CCT AGG AAA CTA A33'	1213	Bsu36I
rs27434	Synonymous	F: 5' GAG TGG GAC CTT GTT TCC AA3' R: 5' TCA GTT CAG GAT GGG TCA CA3'	813	HphI
rs27640	Intronic	F: 5' CTT GTT GTG TTG CTG TTT GG3' R: 5' GCA GAG TTT CTG TAA GCC ATGA3'	683	BsmAI
rs27529	Synonymous	F: 5' TGA TGC TAA TTT GCC CCA TC3' R: 5' CAG TGA GAA GCA TTA CCA AGGA3'	841	HhaI
rs26510	Intronic	F: 5' TGA TGC TAA TTT GCC CCA TC3' R: 5' CAG TGA GAA GCA TTA CCA AGGA3'	841	MboI
rs27582	3' UTR	F: 5' CTG GTG TGA ATT GGG AAG AA3' F: 5' GCA ACA TTT GAG AGG GCA GT3'	980	BsmAI

PCR-RFLP: polymerase chain reaction-restriction fragment-length polymorphism; SNP: single-nucleotide polymorphism; bp: base pairs.

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Table 2. Single-marker association analysis of the 6 SNP in *ARTS1* in 471 AS cases and 456 controls. Adjusted p values were obtained by multivariate logistic regression adjusted for age at examination and sex.

dbSNP	Risk Allele	Frequency of Risk Allele		MAF		p	Covariate-adjusted p	OR (95% CI)
		Cases	Controls	Cases	Controls			
rs26653	G	0.62	0.58	0.38	0.42	0.1041	0.1075	1.17 (0.97, 1.42)
rs27434	A	0.51	0.43	0.49	0.43	0.0068	0.00039*	1.38 (1.09, 1.74)
rs27640	T	0.56	0.53	0.44	0.47	0.3660	0.3174	1.14 (0.86, 1.50)
rs27529	G	0.60	0.55	0.40	0.45	0.0293	0.0083*	1.23 (1.02, 1.49)
rs26510	T	0.59	0.55	0.41	0.45	0.0872	0.0577	1.18 (0.98, 1.43)
rs27582	A	0.57	0.53	0.43	0.53	0.3718	0.4635	1.14 (0.86, 1.50)

* Significant after Bonferroni correction. SNP: single-nucleotide polymorphism; AS: ankylosing spondylitis; MAF: minor allele frequency.

trols (55%) (OR 1.23, 95% CI 1.02 ~ 1.49, p = 0.0083, p_c = 0.049) adjusted for age and sex (Table 2).

Identification of 6 haplotypes associated with AS in a Chinese Han group. Tests revealed strong LD (Figure 1) between any 2 of the 5 SNP (rs27653, rs27434, rs27640, rs27529, rs27510), while rs27582 shared poor LD with any of these 5 SNP (Figure 1). Therefore, haplotype analysis concerning 5 SNP was carried out (rs27653, rs27434, rs27640, rs27529, and rs27510). The result showed that the proportions of haplotypes GATGT (OR 1.68, p = 0.002) and GACGT (p = 0.0004) were higher in AS cases, while the haplotype GGTGT (OR 0.18, p = 3×10^{-7}) was more frequent in controls (Table 3). These findings suggested that haplotypes GATGT and GACGT contributed significant risk effects, while GGTGT is a protective haplotype.

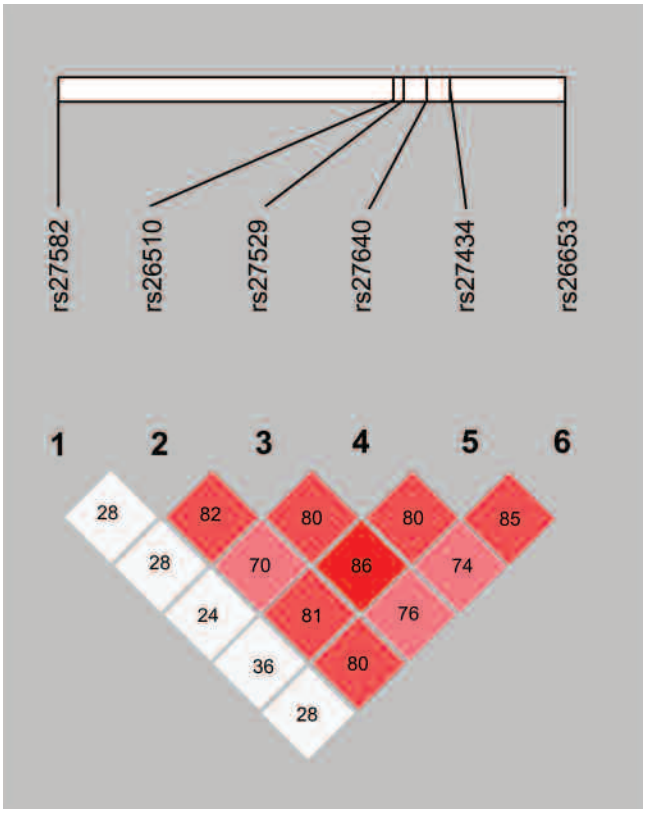

Figure 1. Linkage disequilibrium patterns among 6 SNP: rs26653, rs27434, rs27640, rs27529, rs26510, and rs27582. The pairwise correlation among the SNP was measured as D' and is shown in the diamonds.

Correlations between 6 SNP and clinical measurements. Using multivariate logistic regression with age and sex as covariates, none of the 6 SNP showed significant association with the clinical measurements of AS (age, sex, family history, onset site, dactylitis, peripheral arthritis, hip joint involvement, iritis, enthesitis, ESR, and CRP) except for the SNP rs27510, which was found to be significantly correlated with onset age in patients with AS (p = 0.0083).

DISCUSSION

It is well accepted that AS is a hereditary disease involving many genes[19]. Although HLA-B27 is the most consistent locus associated with AS, it contributes no more than 50% of the overall genetic risk for AS. Recently, 5 SNP within *ERAP1* locus were found to be associated with AS in US and British cohorts, and 2 of these SNP were replicated in a Korean cohort[8]. However, further validation in other populations is necessary, to determine whether *ERAP1* is the common risk locus for AS.

We determined the SNP in the promoter and exon regions of *ERAP1* in a screening sample of Han Chinese by direct sequencing, and performed association tests among the SNP. Six SNP showing suggestive significance were subjected for validation in the same ethnic Han Chinese population of a larger sample size. Two novel SNP within the exon regions of *ERAP1*, rs27434 and rs27529, revealed significant associations with AS, with OR of 1.38 and 1.2, respectively, suggesting that *ERAP1* might be associated with AS in a Han Chinese population. Similarly, another 2 SNP in *ERAP1* had been revealed to be associated with AS in a Han Chinese population (rs27980, p = 0.0048; rs7711564, p = 0.0081). These findings were consistent with the previous report that *ERAP1* or *ARTS-1* was associated with AS in mainland US and British cohorts, indicating that the association of *ERAP1* with AS was replicated in different ethnic populations. On the other hand, the SNP of top significance were different

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Table 3. Significantly associated haplotypes for the 6 SNP. All haplotypes with a frequency < 0.03 were ignored in analysis. Loci chosen for analysis: rs26653, rs27434, rs27640, rs27529, rs26510, and rs27582.

Haplotype	Case, %	Control, %	Chi-square	Fisher's p	OR (95% CI)	PAR, %
GGTGT	2.9	13.5	26.280	3.07e-007*	0.183 (0.090, 0.374)	–4.3
GATGT	42.7	31.4	9.475	0.0021*	1.684 (1.207, 2.349)	4.3
GACGT	3.6	0.0	12.516	0.0004*	—	1.2
GGCAC	5.1	7.6	2.064	0.1508	0.631 (0.335, 1.188)	—
CGTAC	3.7	1.8	2.121	0.1453	2.037 (0.768, 5.407)	
CGCAC	26.7	29.0	0.675	0.4113	0.864 (0.610, 1.224)	

* Significant after Bonferroni correction ($p < 0.00833$). SNP: single-nucleotide polymorphism; PAR: population-attributable risk.

among studies of different populations, suggesting the existence of allelic heterogeneity for *ERAP1* among populations with different ethnic backgrounds. In the Chinese population, risk allele A of SNP rs27434 contributed to 7.4% of the overall genetic risk in AS, which would be a promising target for further study of AS pathogenesis.

Our results indicated that the SNP rs27510 in *ERAP1* was correlated with age at onset in patients with AS, while none of the other 5 associated SNP was correlated with peripheral symptoms or disease activity indices or other measurements. This might suggest the SNP rs27510 in *ERAP1* was associated with age at onset in patients with AS.

However, the power to detect association with an OR of 1.5 is less than 80%. Although this will not affect our conclusions of positive association, the lack-of-association results regarding clinical measurements need to be interpreted with caution.

Single-nucleotide variants in *ERAP1* might have direct functional implications in the molecular pathogenesis of AS. The *ERAP1* gene is located on chromosome 5 and encodes a protein required for shedding of type II interleukin 1 decoy receptor (IL-1RII) and IL-6 receptor[7]. IL-1 is a proinflammatory cytokine that plays an important role in inflammation, host defense, and immunity as well as the pathogenesis of AS[20]. Proteolytic cleavage of the extracellular domain of the IL-1RII generates soluble IL-1-binding proteins that prevent excessive bioactivity by binding free IL-1[7]. Moreover, IL-6 exerts functions through its receptor complex and could induce the expression of an IL-1 receptor antagonist; and stimulates shedding of TNFR1 to a soluble TNF-binding protein[13]. Therefore, the involvement of *ERAP1*, IL-6 signaling, and TNF may be another way that ERAP1 participates in the inflammation process in AS, since TNF-α is a crucial inflammation mediator and has been considered as an inflammatory factor of pathogenesis in AS[21].

Our study provided evidence that *ERAP1* is associated with AS in a Chinese population. With the evidence of previous reports, the study suggested that *ERAP1* is the common susceptibility gene conferring risk of AS, and the pathogenetic mechanism involved might be similar in a general population. However, as indicated by the previous studies, the associated SNP are likely tagging the causative variant(s) that lie within the *ERAP1* locus. Further study is needed to determine the causative variant of *ERAP1* that contributes to the susceptibility of AS. Although discovery of *ERAP1* susceptibility in AS has been a novel breakthrough in genetic studies of AS since the 1970s, when HLA-B27 was identified as a risk locus, further investigations are needed to reveal the underlying molecular mechanisms leading to AS.

ACKNOWLEDGMENT

We thank all the patients with AS and the healthy subjects for their cooperation.

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