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Association between Endoplasmic Reticulum Aminopeptidase-1 (ERAP-1) and Susceptibility to Ankylosing Spondylitis in Iran

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ABSTRACT

Ankylosing Spondylitis (AS) is an inflammatory arthritis, which affects mainly spine and sacroiliac joints. According to recent studies, ERAP1 is the second most common candidate gene for AS susceptibility after HLA-B27. The aim of this study was to determine the association of ERAP1 gene polymorphisms with AS in Iranian population.

The study group comprised 387 Iranian AS patients and 316 healthy controls from Iran. Using Real Time PCR allelic discrimination method, we genotyped four SNPs (rs30187, rs469876, rs13167972 and rs27434) of ERAP1.

We found that rs30187 and rs27434 were significantly associated with AS in Iranian population ($P=6\times10^{-5}$, $P=7\times10^{-3}$, respectively). The rs30187 T/T genotype was associated with AS compared with C/C genotype ($P=1.5\times10^{-5}$). The rs27434 G/G genotype was inversely associated with AS ($P=5\times10^{-3}$). Two specific haplotypes including: rs30187/ rs469876/ rs13167972/ rs27434 TAAA and CAGG were associated with increased and decreased risk of AS in Iranian population, respectively.

These results indicated that ERAP1 SNPs and haplotypes were associated with AS in Iranian population.

Keywords: Ankylosing Spondylitis; Endoplasmic Reticulum Aminopeptidase-1; Single Nucleotide Polymorphism;

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INTRODUCTION

Ankylosing Spondylitis (AS) is an inflammatory rheumatic disease that predominantly affects the spine

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and might be associated with peripheral arthritis.[1, 2] Predisposition of AS is under the effect of genetic and environmental factors.[1] AS has been strongly associated with Major Histocompatibility Complex (MHC), especially HLA-B27.[1-4] Despite the fact that ~ 90% of AS patients express HLA-B27, only 1-5% of HLA-B27 positive individuals ever develop AS conforming strong statistical evidence suggesting that there exists more associating genes with the onset of AS.[5-7] In the past few years, two genome-wide association studies suggest that non-MHC loci including interleukin-1 (IL-1) gene cluster and IL-23 receptor (IL-23R), attribute to AS susceptibility.[4, 8-10] However, less associations have been identified for CYP2D6 (cytochrome P450) and TNFR1 with AS.[11] Endoplasmic Reticulum Aminopeptidase-1 (ERAP-1) is another non-MHC AS associating gene, located on chromosome 5,which regulates shedding of Tumor Necrosis Factor Receptor 1 (TNFR1), therefore, ERAP1 is also called ARTS1.[10] ERAP1 has the second strongest association with AS, with an attributable risk of 26%, while this attributable risk is about 50% for HLA-B27.[2, 10, 12] ERAP1 has two major functions: 1- Trimming N-terminus of peptides in endoplasmic reticulum, when peptides bind to class I HLA molecules and present on cell-surface of T cells.[13] 2- Trimming of cytokine receptors that has been expressed on cell-surface.[14] The association of AS with ERAP1 polymorphism was confirmed in a North American Caucasian replication cohort study.[10]

The association between ERAP1 and AS supports arthritogenic peptide hypothesis. This hypothesis defines the beginning of disease by the presentation of a peptide on the surface of APCs as a result of the HLA-B27 molecules. Therefore AS has been associated with differences in antigen-processing machinery (APM), referred to differences in the HLA-B27 peptide-binding repertoire.[15, 16]

This is the first Iranian ERAP1 replication study with a relatively large sample size. The aim of this study is to determine the role of ERAP1 in susceptibility of AS and examination of the genetic association with four SNPs in ERAP1.

PATIENTS AND METHODS

Patients and Controls

We studied 387 patients suffering from AS whose diagnosis satisfied the modified New York Criteria 1984.[17] The diagnosis of AS was established in all patients by a qualified rheumatologist. AS patients were recruited from Rheumatology Research Center of Tehran University of Medical Sciences, Shariati hospital and Iranian Association of AS. The patient group included 315 men and 72 women, with mean (±SD) age of 38.2 (±10.6) years, disease duration of 15.5 (±9.4) years and 73.5% of whom were HLA-B27 positive. Altogether, 316 (231 men and 85 women) healthy unrelated controls with mean age of 34.1 (±10.5) years, frequency matched by age (±4 years), gender, and ethnicity without personal or family history of autoimmune or rheumatic diseases were included. The study was approved by the Ethical committee of Tehran University of Medical Sciences and informed written consents were obtained from each individual (cases and healthy controls).

ERAP1 Genotyping

Genomic DNA of cases and controls were isolated from peripheral blood leucocytes, using proteinase K phenol-chloroform extraction procedure.[18] Samples were genotyped for 4 SNPs (rs30187, rs469876, rs13167972, and rs27434), which were also used in the two previous studies (Table 1).[10, 19] Real-Time PCR allelic discrimination TaqMan genotyping assays (Applied Biosystems, Foster City, USA) were used for genotyping, which was performed by an ABI 7300 Real-Time PCR system according to the Applied Biosystem protocol. The allelic call was performed by the analysis of allelic discrimination plots using, ABI SDS V 1.4 software.

Table 1. characteristics of SNPs genotyped in the ERAP1 gene

SNP	Minor Allele	Position	Location/Exon	Amino Acid Change
rs30187	T	96150086	11	Lys528Arg
rs469876	G	96147162	IVS13+85	Intronic
rs13167972	G	96142564	20	Non-coding
rs27434	A	96155268	6	Ala356

Table 2. Frequencies of ERAP-1 genotypes and associations with AS

Genotypes	Cases (n=387) (%)	Controls (n=316) (%)	OR (95% CI)†	*P*. value
rs30187				
C/C	88 (23.1)	104 (33.3)	1 (referent)	
C/T	190 (49.9)	163 (52.2)	1.38 (0.97-1.96)	
T/T	103 (27.9)	45 (14.4)	2.70 (1.72-4.25)	1.51×10^{-5}
χ^2 (2df)‡	19.45 (5.97×10^{-5})			
C/T or T/T vs. C/C (single locus model)			1.66 (1.19-2.33)	3×10^{-3}
C/T or T/T vs. C/C (four locus model)			1.4 (0/92-2.1)	
rs469876				
A/A	231 (65.8)	179 (60.3)	1 (referent)	
A/G	107 (30.5)	102 (34.3)	0.81 (0.58-1.13)	
G/G	13 (3.7)	16 (5.4)	0.63 (0.29-1.34)	
χ^2 (2df)	2.53, P=0.28			
A/G or G/G vs. A/A (single locus model)			0.79 (0.57-1.09)	
A/G or G/G vs. A/A (four locus model)			0.82 (0.55-1.22)	
rs13167972				
A/A	102 (36.7)	81 (29.0)	1 (referent)	
A/G	132 (47.5)	151 (54.1)	0.69 (0.48-1.01)	
G/G	44 (15.8)	47 (16.8)	0.74 (0.45-1.23)	
χ^2 (2df)	3.78, P=0.15			
A/G or G/G vs. A/A (single locus model)			0.71 (0.49-1.01)	5.5×10^{-2}
A/G or G/G vs. A/A (four locus model)			0.65 (0.45-0.95)	
rs27434				
A/A	55 (14.4)	28 (8.9)	1 (referent)	
A/G	188 (49.3)	141 (44.6)	0.68 (0.41-1.12)	
G/G	138 (36.2)	147 (46.5)	0.48 (0.29-0.80)	5×10^{-3}
χ^2 (2df)	9.80, $P=7\times10^{-3}$			
A/G or G/G vs. A/A (single locus model)			0.58 (0.36-0.93)	2.5×10^{-3}
A/G or G/G vs. A/A (four locus model)			0.65(0.38-1.13)	

* Frequencies are based on: rs30187 on 381 cases and 312 controls, rs469876 based on 351 cases and 297 controls, rs13167972 based on 278 cases and 279 controls and rs27434 based on 381 cases and 316 controls.†OR (odds ratios) and 95% CI (confidence intervals) were estimated using logistic regression models for each locus separately (single locus model) and adjusting for genotypes at all four loci (four locus model) as indicated.‡χ^2Statistic shown.

Analysis

The Hardy-Weinberg equilibrium was examined for each SNP separately in case and control groups using Pearson χ^2 test in genetics package1 (α=0.05).

However, when required, the Bonferroni correction method was used in multiple statistical testing (i.e. *P. value* was set to <0.01) to declare a statistically significant results. The χ^2 or Fisher's exact test was used for testing the association between genotype frequencies and case/control groups at each SNP separately. Finally, the multivariable logistic regression was performed for investigating the overall relationship between SNPs and AS disease. The Odds ratios (ORs) and their associated 95% confidence intervals (CIs) were calculated.

Haplotypes and their corresponding posterior probabilities were estimated using EM algorithm. Differences between such probabilities were tested consequently. All statistical analyses were performed using, statistical software R version 2.13.[20]

RESULTS

Results of testing HWE (not shown here) were all non-significant (P>0.05) indicating that SNPs under investigation are all in Hardy-Weinberg equilibrium. Table 2 shows the frequency of ERAP-1 polymorphisms in case and control groups.

Table 3. Association of ERAP1 haplotypes with AS in Iranian population

	Estimated Haplotypes				Frequencies		
	rs30187	rs469876	rs13167972	rs27434	Hap.freq (Case)	Hap.freq (Control)	*P. value*
1	C	A	A	G	O.089	0.098	0.672
2	C	A	G	A	-	0.002	-
3	C	A	G	G	0.202	0.269	0.011
4	C	G	A	G	0.181	0.211	0.182
5	C	G	G	G	0.007	0.016	0.321
6	T	A	A	A	0.226	0.160	0.009
7	T	A	A	G	0.112	0.088	0.185
8	T	A	G	A	0.165	0.150	0.519
9	T	A	G	G	0.017	0.003	0.076
10	T	G	A	G	-	0.003	-

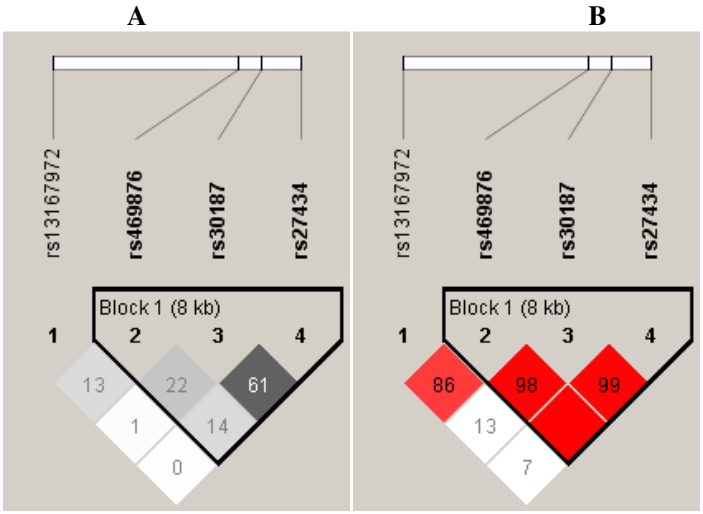

Figure 1. Pairwise D′ and r^2 LD plots of ERAP1 SNPs in the association analysis. A. The plot shows r^2×100 value as pairwise measure of LD B: The plot shows pairwise D′ measure of LD. Numbers inside squares represent intensity of linkage disequilibrium (r^2 and D′) between every two SNPs.

The rs30187, was significantly associated with AS (χ^2 = 19.45; *P*=5.97×10^{-5}); the rs30187T/T genotype was significantly associated with AS compared to the rs30187C/C genotype (OR = 2.70; *P*<0.01). Carriage of T allele was associated with AS in single locus model (OR=1.66; *p*=0.003). After adjusting for confounding effects of other polymorphisms, the apparent association was not statistically significant (OR = 1.40; *P*=0.12). The rs469876 and rs13167972 were not associated with AS. Genotype frequencies at rs27434 differed between cases and controls (χ^2=9.80; *P*=0.007). The rs27434 G/G genotype was inversely associated with AS comparing with the A/A genotype (OR = 0.48; *p*=0.005). Carriage of the rs27434 G allele was inversely associated with AS in single locus model (OR=0.58; *P*=0.0025). The apparent association was not significant after adjusting for confounding effects of other polymorphisms (OR=0.65; *P*=0.13).

Based on Table 3 of haplotype analysis, the most prevalent haplotype is rs30187/ rs469876/ rs13167972/ rs27434- CAGG in the control group and rs30187/ rs469876/ rs13167972/ rs27434- TAAA in AS group with 27% and 23% of posterior probabilities, respectively. The estimated prevalence of rs30187/ rs469876/ rs13167972/ rs27434- TAAA haplotype in AS group was markedly higher in patients compared to the control group (23% vs. 16%, *P*=0.009). However, the estimated prevalence of rs30187/ rs469876/

rs13167972/ rs27434- CAGG was lower in AS group in comparison to the control group (P=0.011).

The computation of linkage dsequilibrium (LD) between ERAP1 SNPs was usig of D′ and r^2. Pairwise D′ and r^2 of four SNPs and haplotype analysis were accomplished, using Haploview V.4.2 software. Three of these SNPs in ERAP1 gene (rs469876, rs30187 and rs27434) were in strong LD (D′>98 and r^2= 61 and 22 percents). These three SNPs formed an LD block in 8kb region (Figure 1).

DISCUSSION

AS is a chronic inflammatory autoimmune disease of the axial skeleton identified by back pain and stiffness of the spine. AS is a multi-factorial disease, so genetic and environmental factors have important roles in its pathogenesis.[1, 21, 22]

After completion of two important projects (Human genome and international Hapmap projects), several techniques were developed to discover genetic associations with human diseases.[23, 24] Since 2006, many genome wide association and replication studies were accomplished and nearly 100 loci for more than 40 common diseases were identified.[23, 24] There are few genome wide association studies that introduce several genes and loci associated with AS such as ERAP1, IL23R, IL1R2 and ANTXR.[3, 4, 10, 25]

ERAP1 is a member of M1 family of metalopeptidases,[1] which has enzymatic function in antigen presentation and inflammatory responses. ERAP1 has two main functions in regulation of immune and inflammatory processes: 1- N-terminus peptide trimming on endoplasmic reticulum for antigen presentation by MHC-I molecules on cell surface. 2- Ectodomain cleavage of pro-inflammatory cytokine receptors such as TNFR1, IL6Rα, and IL1R2.[13, 14, 26-28] Therefore, ERAP1 can be considered potentially an attractive candidate gene for AS.

HLA-B27 with an attributable risk of about 50% has the highest association among AS patients. According to relatively lower frequency of HLA-B27 in Iranian patients, [29-32] non-MHC genes seem to play more important roles in the disease onset and pathogenesis of AS compared to other studied populations. Thus, as previously mentioned, the ERAP1 functions make it a good candidate gene for studies in Iranian population.

The association between ERAP1 gene and AS was replicated with strong significance in the Iranian AS patients. We studied rs30187, rs469876, 13167972 and rs27434 in ERAP1 gene in our Iranian population samples. The rs30187 and rs27434 were both significantly associated with AS susceptibility.

According to Table 2, rs30187 showed an overall association with disease susceptibility in Iranian AS patients (χ2=19.45, P=5.97× 10^{-5}). The rs30187 T/T genotype was associated with susceptibility to AS compared to C/C genotype (OR=2.70, P=1.51×10^{-5}). Indeed, viewed as a positive risk factor, carriage of rs30187T was associated with about two-times higher risk of AS than CC genotype in single locus model (OR=1.66, P=3×10^{-3}). After adjusting for confounding effects of the other three polymorphisms, data revealed that rs30187 was not associated with AS in four-locus model. It seems that this occurs because of strong LD between rs30187 and other polymorphisms (Fig 1). According to genome wide association and replication studies, rs30187 has been also associated with AS in all populations except Hungarian population.[3, 4, 33-35]

According to Goto et.al, rs30187 has an important role in reducing the activity of EARP1 and also in substrate affinity.[3, 36] The rs30187 codes Arg 528 located in the mouth of the enzyme substrate pocket which perhaps is the cause of the lower activity of such allele (i.e. rs30187) compared to the wild type gene.[3, 37] Rs30187 allele with slower rates of peptide trimming than wild-type ERAP1 (~40%) is a protective allele because of its lower function.[4]

Overall, the rs27434 was significantly associated with AS, and rs27434 G/G genotype was inversely associated with the disease compared to A/A genotype (OR=0.48, P=0.005). The rs27434 A/A genotype was associated with about two fold higher risk of AS than carriage of G allele. The rs27434 was associated with AS in Korean and Chinese populations. The rs27434 also showed a great association with AS in TASC study.[23, 25, 34, 38, 39] This SNP also showed a strong association with ERAP1 gene expression.[19, 37]

Moreover, to the best of our knowledge, this study is the first to report the specific ERAP1 haplotypes, which have protective and susceptible roles in AS. One of these significant haplotypes, rs30187/ rs469876/ rs13167972/ rs27434- TAAA was associated with susceptibility to AS and the other haplotype, CAGG, had a protective role in AS disease.

Our study indicated that ERAP1 SNPs and haplotypes were associated with AS in Iranian population. In order to determine the possible association of ERAP-1 SNPs with AS, further studies will be needed.

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