Molecular analysis of ERAP1 Allelic Variations in patients with Ankylosing Spondylitis

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ABSTRACT

Ankylosing spondylitis (AS) is a group chronic inflammatory arthritis termed seronegative spondyloarthropathies. It typically affects the joints of the spinal and axial skeleton and exhibits genetic factors such as HLA-B27 and ERAP1. Among the non-HLA loci, the strongest association was observed for the ERAP1 of SNPs. We have determined the frequencies of ERAP1 allelic variants and genotypes for 3 SNPs in AS patients and healthy individuals from the Iranian population. We implemented the SSP-PCR method for genotyping of 160 AS patients and 160 healthy controls from the Iranian population. Significant differences in allele's frequencies within patients vs control cohort were shown for 3 SNPs including rs30187, rs2287987, and rs10050860 under investigation. The ERAP1 gene polymorphism might be a risk factor in the pathogenesis of AS. In contrast, ERAP1 gene polymorphisms may serve a protective role in AS.

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INRTODUCTION

Ankylosing spondylitis (AS) is a group chronic inflammatory arthritis termed seronegative spondyloarthropathies that mainly affects the spine and pelvis in young men. Chronic inflammation in joints causes the change of joint architecture with bone regenerations. The unique structural alters of syndesmophyte formation, and ankylosis of the vertebrae are the primary causes of early severe work disability of AS patients with disease progressions. Recently genome-wide association studies have provided novel insights into the AS immunopathogenesis, such as new variants responsible for AS predisposition other than HLA-B27. Among the non-HLA loci, the strongest association was observed for the ERAP1 gene. ERAP1 belongs to the M1 family of aminopeptidases that plays two roles in of immune response. One of the roles of ERAP1 is a "molecular ruler," and play in the N-terminal proteolysis of peptides and other role known as the "receptor sheddase," and cleavage of cell surface receptors for proinflammatory cytokines. This study we have determined the frequencies of ERAP1 allelic variants and genotypes 3 SNPs including rs30187, rs2287987, and rs10050860 and risk of AS in the Iranian population(1-2).

METHODS AND MATERIALS

This study has been performed from November 2015 and April 2017 on 160 patients [140 (87.5%) males and 20 (12.5%) females, range 23-62 years] with AS diagnosed according to the modified New York Criteria. Patients with AS were employed at the Immunology Research Center of Tabriz University of Medical Sciences. We applied BASDAI to assess the disease activity of the AS patients enrolled. The control group composed of 160 age, gender, and ethnically matched healthy individuals (75% males versus 25% females) without personal or family history of AS and other autoimmune diseases.

METHODS AND MATERIALS

Sample collection and DNA Extraction: Peripheral blood mononuclear cells (PBMCs) were prepared from fresh whole blood tubes containing EDTA and using Ficoll isolated PBMCs. Genomic DNA samples of AS and healthy controls were extracted by using the QIAamp DNA Mini Kit.

Single Specific Primer-Polymerase Chain Reaction: We implemented the SSP-PCR system for genotyping of AS patients and healthy controls from the Iranian population. Forward and reverse primers were designed for rs30187, rs2287987, rs10050860 and β-actin using OLIGO7 Software and purchased from Metabion company (Germany).

RESULTS

A. Associations between the ERAP1 variants and AS

Data analysis showed there was a significant association between ERAP1 variants and risk of AS. Beside for three SNPs, there were significant correlations between rs30187T allele (P=0.037; OR=0.707; 95% CI=0.516-0.968), rs2287987C allele (P=0.015; OR=1.60; 95% CI=1.10-2.32), rs10050860T (P=0.025; OR=1.57; 95% CI=1.07-2.33) and an increased risk of AS. The genotype distributions of the rs30187 and rs10050860 in the ERAP1 were in Hardy-Weinberg equilibrium (HWE) in the AS groups. The results of the genotypic and the minor allelic frequency analysis are shown in Table 1.

dbSNP		Frequency						
		AS (%)	Controls (%)		AS (%)	Controls (%)	Genotypic	Allelic
	Genotype	N=160	N=160	Allele	N=160	N=160	P-values	P-values
rs30187 [C/T]	CC	55 (34)	32 (20)	T (MAF)	121 (39)	148 (46)	0.015*	0.037*
	CT	89 (56)	108 (68)	C	199 (61)	172 (54)		
	TT	16 (10)	20 (12)	CC+TT *vs.* CT	OR (95%CI) : 0.604 (0.383- 0.951)		0.029*	
				CT+TT *vs.* CC	OR (95%CI) : 2.09 (1.26-3.47)		0.008*	
				CT+CC *vs.* TT	OR (95%CI) : 0.778 (0.387-1.56)		0.596	
HWE P-values		0.238	0.006*	OR (95%CI) : 0.707 (0.516-0.968)				
rs2287987 [C/T]	CC	27 (14)	10 (6)	C (MAF)	89 (22)	62 (19)	0.011*	0.015*
	CT	35 (25)	42 (26)	T	231(78)	258 (81)		
	TT	98 (61)	108 (68)	CC+TT *vs.* CT	OR (95%CI) : 0.787 (0.470-1.31)		0.443	
				CT+TT *vs.* CC	OR (95%CI) : 3.04 (1.42-6.52)		0.005*	
				CT+CC *vs.* TT	OR (95%CI) : 0.761 (0.481-1.20)		0.293	
HWE P-values		0.0001*	0.412	OR (95%CI) : 1.60 (1.10-2.32)				
rs10050860 [C/T]	CC	91(57)	108 (68)	T (MAF)	79 (12)	55 (17)	0.046	0.025*
	CT	59 (37)	49 (31)	C	241 (88)	265 (83)		
	TT	10 (6)	3 (1)	CC+TT *vs.* CT	OR (95%CI) : 1.32 (0.831-2.10)		0.287	
				CT+TT *vs.* CC	OR (95%CI) : 0.635 (0.403-1.01)		0.065	
				CT+CC *vs.* TT	OR (95%CI) : 3.48 (0.942-12.92)		0.086	

Table 1. The distribution of allele and genotype frequencies of ERAP1 gene polymorphism in AS patients and healthy controls under Co-Dominant, Recessive and Dominant models.
MAF: minor allele frequency, HWE: Hardy Weinberg Equilibrium, AS: ankylosing spondylitis, OR: odds ratio, CI: confidence interval, dbSNP: database of single nucleotide polymorphisms, *P<0.05.

B. ERAP 1 haplotypes are associated with AS susceptibility

We subsequently investigated the effect of ERAP1 SNP haplotypes on AS susceptibility. the TTC frequency was found to be significantly associated with AS risk ($p < 0.00007$, OR = 0.51). The frequency of the TTC haplotype was to be lower in AS patients as compared to the healthy individuals, suggesting that the haplotype TTC is a risk marker for AS (Table 2). Also did not observe any correlation between these three genetic variants with disease activity.

Block 1 Haplotypes				Frequencies				
Row	rs30187	rs2287987	rs10050860	Hap Freq. (Case) N (%)	Hap Freq. (Control) N (%)	OR* (95% CI*)	χ^2	*P*
1	C	C	T	11 (3.4)	13 (4)	0.84 (0.37-1.90)	0.173	0.677
2	C	T	C	90 (28.1)	84 (26.2)	1.09 (0.77-1.55)	0.284	0.593
3	C	T	T	48 (15)	33 (10.3)	1.53 (0.95-2.46)	3.18	0.074
4	**T**	**T**	**C**	**88 (27.5)**	**136 (42.5)**	**0.51 (0.36-0.71)**	**15.82**	**0.00007**
5	C	C	C	48 (15)	37 (11.5)	1.34 (0.85-2.13)	1.64	0.2
6	T	C	C	15 (4.6)	9 (2.8)	1.69 (0.73-3.94)	1.55	0.211

Table 2. Overall haplotype associations of the SNPs according to Haploview.
*OR; odds ratio, 95% CI; 95% confidence interval

DISCUSSION

Previous studies have distinctly presented the importance of the ERAP1 variants in the immunopathophysiology of AS. Despite comprehensive studies, the immunopathogenesis of AS remains not entirely understood. Recently, Liye Chen *et al.* exhibited that the protective allele at rs30187 to be associated with reduced HLAB27 free heavy chain expression and expansion Th17 response, which increased on silencing ERAP1 (3). Beside ERAP inhibition may suggest a novel therapeutic option for AS given that ERAP1 inhibitors are recently in development. This study is the first report about the association between these ERAP1 SNPs and AS-risk in Iranian population. In conclusion, our study suggests that rs30187, rs2287987, and rs10050860 may be associated to AS predisposition. In contrast, ERAP1 gene polymorphisms may serve a protective role in AS.

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