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The Open Rheumatology Journal, 2017, *11*, 1-9

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The Open Rheumatology Journal

Content list available at: www.benthamopen.com/TORJ/

DOI: 10.2174/1874312901711010001

RESEARCH ARTICLE

Association of *ERAP1*, *IL23R* and *PTGER4* Polymorphisms with Radiographic Severity of Ankylosing Spondylitis

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Received: June 08, 2016 Revised: December 14, 2016 Accepted: January 04, 2017

Abstract:

Background:

Radiographic severity of ankylosing spondylitis (AS) shows such great variance that some patients never develop syndesmophytes throughout the entire disease span, whereas some develop bamboo spine relatively early.

Objective:

To study the association between *ERAP1*, *IL23R* and *PTGER4* single nucleotide polymorphisms (SNPs) and radiographic severity in AS patients.

Methods:

rs27044 and rs30187 (*ERAP1*), rs11209032 (*IL23R*) and rs10440635 (*PTGER4*) SNPs were genotyped in 235 AS patients fulfilling the modified New York criteria. Patients were classified as mild- and severe-AS according to modified Stoke AS spinal score (mSASSS). Mild-AS is defined as having mSASSS of "0" following at least 10 years of disease duration. Severe-AS is defined as having mSASSS of >20 (patients with mild vertebral changes (*i.e.* squaring or erosions) were omitted for clear stratification) regardless of disease duration.

Results:

The genotype distributions and allele frequencies of *ERAP1* rs27044 and rs30187, *IL23R* rs11209032 and *PTGER4* rs10440635 SNPs were similar in mild- (n=171, mSASSS=0, 55.6% HLA-B27 positive) and severe-AS patients (n=64, mSASSS=48.5±17.8, 73.4% HLA-B27 positive). After adjustment for clinical differences between groups (gender, disease duration, HLA-B27 and smoking status) by logistic regression analysis, none of the alleles in the investigated SNPs were found to be associated with radiographic severity of AS.

Conclusion:

In radiographically well-categorized AS patients, *ERAP1* rs27044 and rs30187, *IL23R* rs11209032 and *PTGER4* rs10440635 SNPs are not found to be associated with radiographic severity of AS.

Keywords: Ankylosing spondylitis, radiographic severity, *ERAP1*, *IL23R*, *PTGER4*.

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INTRODUCTION

Ankylosing spondylitis (AS) is a polygenic chronic inflammatory disease predominantly affecting the axial skeleton which leads to the new bone formation and disability. However, the link between new bone formation and inflammation in AS has not yet been fully understood [1, 2]. Furthermore, it is clearly demonstrated that new bone formation shows great variance in AS patients so that some patients never develop syndesmophytes throughout the entire disease span, whereas some rapidly develop spinal fusion [3, 4]. The extent of new bone formation is thought to be determined primarily by genetic factors with the contribution of environmental and acquired factors [5]. Despite growing evidence in the last decade for a genetic predisposition of the new bone formation, controversy remains for the individual genetic determinants of radiographic severity in AS. Recent genome-wide association studies have provided new insights into the AS pathogenesis, such as identification of new susceptibility genes other than HLA-B27 [6 - 8]. The strongest associations for AS susceptibility were identified in *ERAP1* and *IL-23R* genes, contributing to 26% and 9% of AS risk, respectively [6]. *ERAP1* gene product has an important role in antigen processing and regulates trimming of N-terminally extended peptides to their final epitopes, which are then presented by the major histocompatibility complex class I molecules [9, 10]. *IL-23R* gene encodes the receptor of IL-23, the key cytokine in the differentiation of naive CD4+ T cells into IL-17 producing T helper cells [11]. Polymorphisms in both genes have potential implications on inflammatory pathways in AS [12]. A strong association between the prostaglandin E receptor 4 (*PTGER4*) gene and AS susceptibility has also been reported [8]. The receptor encoded by this gene can activate T-cell factor signaling, mediate PGE2-induced expression of early growth response 1, and regulate the level and stability of cyclooxygenase-2 mRNA [13]. As a component of the "mechanostat" which regulates the anabolic bone response to physical stress at sites such as the enthesis, *PTGER4* single nucleotide polymorphisms (SNP) may have a role in the new bone formation of AS [8].

The associations of these gene SNPs with AS have been well-established and replicated in multiple studies [14 - 17]. However, the contribution of these genes to radiographic severity/progression in AS has been investigated in only three studies in which radiographic severity or progression has been defined vaguely and differently [17 - 19]. Radiographic progression in AS is a very slow process [3, 4] in which determinants remain unclear. Therefore, in the present study, patients with AS were divided into two highly distinct groups: 1. Mild-AS defined as no evidence of axial radiographic progression following at least 10 years of disease duration, 2. Severe-AS defined as the presence of multiple bridging or syndesmophytes regardless of the disease duration. The primary aim of the study was to investigate the possible associations between *ERAP1, IL23R,* and *PTGER4* gene polymorphisms which are linked to alterations in inflammatory pathways and new bone formation, and radiographic severity of AS. A second objective was to identify clinical characteristics associated with severe radiographic AS.

PATIENTS AND METHODS

Study Design and Patients

This cross-sectional study comprised of a total of 235 patients fulfilling the 1984 modified New York criteria for AS [20]. Patients were unrelated Caucasians recruited from a single center. Patients' demographics, disease characteristics including peripheral arthritis, extra-articular manifestations, HLA-B27 status, joint and spinal surgery history, and previous medications were collected. All patients had lumbar and cervical vertebral radiographs in anteroposterior and lateral planes taken within the last six months prior to the study entry. Patients who had other concomitant inflammatory diseases or did not fulfill the mild or severe radiographic AS criteria defined in the following section (*i.e.* intermediate changes) were excluded. The study was approved by the Marmara University local ethics committee (B.30.2.MAR.0.01.02/AEK/196 Date: 23.02.2012), and informed consent was obtained from all patients according to the Declaration of Helsinki before study entry.

Radiographic Scoring and Severity Classification

Lumbar and cervical radiographs were scored using modified Stoke AS spinal score (mSASSS) system [21] by 2 rheumatologists (GO, PA) blinded to clinical data. Inter-observer reliability was good with a Cohen's kappa of 0.93.

Currently there is no well-defined uniform definition for radiographic severity of AS in the literature. In this study, we classified the patients as mild radiographic AS and severe radiographic AS according to mSASSS scores. Mild-AS was defined as having an mSASSS of "0" following at least 10 years of disease duration. Patients with an earlier phase of axial involvement, *i.e.*, patients with at least one vertebral corner scored as "1", were excluded from the mild group.

Severe-AS was defined as having an mSASSS of >20 (vertebral corners scored with "1" were not added to the total score, *i.e.*, only syndesmophytes and bridging were scored to include patients with extensive radiographic progression) regardless of disease duration. Because the first 10 years is the period in which the majority of radiographic progression occurs [22], having no radiographic progression (mSASSS=0) after 10 years of disease was considered mild radiographic AS. On the other hand, mSASSS >20 was chosen as severity cut-off since a score of >20 without counting "1" requires bridging of at least 3 vertebrae or syndesmophytes at about one-third of the scored vertebral sites which is associated with significant functional disability and predicts further radiographic progression. A total of 430 patients' radiographs were scored. Patients with only squaring, sclerosis or erosion (n=119) and patients with syndesmophytes but mSASSS <20 (n=76) were excluded. Sixty-four and 171 patients were classified as severe and mild radiographic AS, respectively and included in the study.

SNP Selection, DNA Extraction and Genotyping

rs27044 and rs30187 (*ERAP1* gene), rs11209032 (*IL23R* gene) and rs10440635 (*PTGER4* gene) SNPs were genotyped. The rationale for selecting these SNPs is that they are the most commonly and highly associated SNPs in AS susceptibility in Caucasian populations with odd ratios (OR) of 1.40, 1.33, 1.70 and 1.20, respectively [7, 8, 17, 23, 24].

Venous blood samples (2 mL) were collected from study participants into EDTA-containing tubes and stored at +4 ^{o}C until analysis. Genomic DNA was isolated from peripheral blood leukocytes using the High Pure Polymerase Chain Reaction (PCR) Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) and used as a template for genotyping procedures. SNPs variant (*IL23R* rs11209032, *PTGER4* rs10440635, *ERAP1* rs30187 and 27044) genotyping was performed by allelic discrimination using specific Taqman Probes (Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 45 cycles of denaturing at 95°C for 15 seconds, and annealing and extension at 60°C for 1 minute. All assays were performed in 96-well plates, including negative template controls.

Statistical Analyses

Continuous variables were presented as mean±SD if variables were normally distributed and as median with interquartile ranges (IQR) if not. The differences in demographic and disease characteristics of mild and severe radiographic AS patients were evaluated using either Chi-square and Student-t test or nonparametric tests (Wilcoxon's signed rank test, Mann- Whitney U test), as applicable. Comparisons of the genotype distributions and allele frequencies were analyzed using the Chi-square test. The Odds ratios (OR) and 95% confidence interval of the relevant SNPs for radiographic severity were calculated by using logistic regression analysis, controlling for gender, disease duration, HLA-B27 and smoking status. The candidate factors associated with radiographic severity of AS identified in univariate analysis with a P value of <0.05 were analyzed using a stepwise multivariable logistic regression model to determine independent factors for severe-radiographic AS. The following variables were included in the analysis: gender, coxofemoral joint involvement, uveitis, smoking, and HLA-B27 status. Hosmer-Lemeshow goodness of fit statistics was used to assess model fit. The level of significance was chosen to be P<0.05.

A priori sample size calculation was performed using the minor allele frequencies described in the European population for the four SNPs included in this study (http://www.broadinstitute.org/mammals/haploreg/haploreg.php), for a desired power of 80% with a significance level of 5%. The minimum required sample size was calculated using the Bioinformatics Institute's Online Sample Size Estimator (http://osse.bii.a-star.edu.sg/calculation1.php) with the assumption of the proportions of risky alleles of 65% and 40% in the severe and mild group, respectively. Severe- to mild-AS patient ratio was set as 1:2 and the sample size calculation yielded at least 46 patients for the severe group and 92 patients for the mild group. Statistical analysis was performed using the SPSS software version 16.0 (SPSS, Chicago, IL).

RESULTS

Characteristics of Study Patients

A total of 235 AS (F/M=97/138, mean age 42.1±11.0 years, mean disease duration 16.5±7.2 years) patients were included in the study. HLA-B27 was positive in 142 (60.4%) patients. Sixty-one (26%) had coxofemoral joint involvement and 94 (40%) patients had any extra-articular involvement, with 37 (15.7%) and 63 (26.8%) patients uveitis and enthesitis were the most common, respectively. Of the 235 patients, 223 (95%) were previously exposed to

nonsteroidal anti-inflammatory drugs (NSAIDs), and 108 (45.9%) patients were on NSAIDs and 88 (37.4%) on tumour necrosis factor-α inhibitors (TNFi) at the time of the study. According to the severity classification by mSASSS, 171 (72.8%) patients were classified as mild-AS and 64 (27.2%) as severe-AS (mean mSASSS 0 *vs.* 48.5±17.8, *P*<0.001). Severe-AS patients were mainly male and significantly older, had longer disease durations, higher acute phase reactants, more frequent extra-articular and coxofemoral joint involvement, HLA-B27 positivity, and TNFi use Table **1**.

Table 1. Demographics and disease characteristics of mild- and severe-AS patients*.

	Mild-AS (n = 171)	Severe-AS (n = 64)	*P* value
Male, n (%)	83 (48.5)	55 (85.9)	<0.001
Age, years	38.7±9.4	51.2±9.8	<0.001
Disease duration, years	14.1±4.8	23.0±8.5	<0.001
Ever-smoked n (%)	42 (24.6)	24 (37.5)	0.049
HLA-B27 positivity, n (%)	95 (55.6)	47 (73.4)	0.013
Enthesitis, n (%)	44 (25.7)	19 (29.7)	0.543
Peripheral arthritis, n (%)	75 (43.9)	31 (48.4)	0.531
Coxofemoral joint involvement, n (%)	35 (20.5)	26 (40.6)	0.002
Uveitis, n (%)	20 (11.7)	17 (26.6)	0.005
TNFi ever-used, n (%)	64 (37.4)	43 (67.2)	<0.001
NSAID current users, n (%)	81 (47.4)	27 (42.2)	0.540
ESR, mm/h, median (IQR)	20 (12-34)	30 (15.7-48)	0.005
CRP, mg/L, median (IQR)	5 (3.4-9.2)	9.6 (3.6-20.1)	<0.001
mSASSS (0-72)	0	48.5±17.8	<0.001

* Values are presented as mean±SD, unless indicated otherwise.

AS= Ankylosing spondylitis; TNFi= TNF inhibitor; NSAID= Nonsteroidal anti-inflammatory drugs; ESR= Erythrocyte sedimentation rate; CRP= C-reactive protein; mSASSS= modified Stokes AS spinal score.

Association of *ERAP1*, *IL23R* and *PTGER4* SNPs on Radiographic Severity

The genotype frequency of the entire AS cohort is shown in Table **2**. There was no significant difference in the genotype distributions of *ERAP1* rs27044 and rs30187, *IL23R* rs11209032 and *PTGER4* rs10440635 SNPs between severe and mild radiographic AS (Table **3**).

Table 2. Genotype frequencies of the 4 SNPs in *ERAP1*, *IL23R* and *PTGER4* in the entire cohort (n=235).

SNP	Gene	Genotype	Frequency
rs27044	*ERAP1*	CC/ CG/ GG	0.48/ 0.46/ 0.06
rs30187	*ERAP1*	CC/ TC/ TT	0.33/ 0.51/ 0.16
rs11209032	*IL23R*	GG/ AG/ AA	0.31/ 0.45/ 0.24
rs10440635	*PTGER4*	GG/ AG/ AA	0.23/ 0.50/ 0.27

Table 3. Genotype distributions of *ERAP1*, *IL23R* and *PTGER4* SNPs and HLA-B27 status of mild and severe radiographic AS patients*.

	Mild radiographic AS (n = 171)	Severe radiographic AS (n = 64)	*P* value
HLA-B27 positivity	95 (55.6%)	47 (73.4)	0.013
***ERAP1* rs27044**			0.69
CC	85 (49.7)	29 (45.3)	
CG	77 (45)	30 (46.9)	
GG	9 (5.3)	5 (7.8)	
***ERAP1* rs30187**			0.66
CC	59 (34.5)	19 (29.7)	
TC	87 (50.9)	33 (51.6)	
TT	25 (15.6)	12 (18.8)	
***IL23R* rs11209032**			0.43
GG	54 (31.6)	18 (28.1)	
AG	80 (46.8)	27 (42.2)	

(Table 3) contd.....

	Mild radiographic AS (n = 171)	Severe radiographic AS (n = 64)	*P* value
AA	37 (21.6)	19 (29.7)	
***PTGER4* rs10440635**			0.62
GG	47 (27.5)	17 (26.6)	
AG	83 (48.5)	35 (54.7)	
AA	41 (24)	12 (18.8)	

*Values are provided as n (%).

The genotype frequencies did not change when mild- and severe-AS patients were categorized by gender or HLA-B27 positivity. Similarly, compound minor allele carrier state (any combination of *ERAP1* SNPs or *IL23R* rs11209032 and *PTGER4* rs10440635 SNPs) did not change significantly among mild- and severe-AS patients. Allele frequencies of the SNPs were also comparable in both mild- and severe-AS patients. After adjustment for clinical differences between the two groups (gender, disease duration, HLA-B27 and smoking status), by logistic regression analysis none of the alleles in the investigated SNPs were found to be associated with radiographic severity of the AS (Table **4**).

Table 4. The distribution of allele frequencies of *ERAP1*, *IL23R* and *PTGER4* SNPs of mild- and severe- radiographic AS patients*.

	Mild-AS (n = 171)	Severe-AS (n = 64)	*P* value	OR (95%CI)
	Allele frequency			
***ERAP1* rs30187**			0.86	0.91 (0.34 to 2.4)
C	59 (34.5)	19 (29.7)		
T†	112 (65.5)	45 (70.3)		
***ERAP1* rs27044**			0.78	1.14 (0.46 to 2.84)
C	85 (49.7)	29 (45.3)		
G†	86 (50.3)	35 (54.7)		
***IL23R* rs11209032**			0.61	1.24 (0.54 to 2.85)
G	54 (31.6)	18 (28.1)		
A†	117 (68.4)	46 (71.9)		
***PTGER4* rs10440635**			0.39	0.68 (0.29 to 1.62)
G	47 (27.5)	17 (26.6)		
A†	124 (72.5)	47 (73.4)		

*Values are provided as n (%).

†Risk allele; OR= odds ratio; CI= confidence interval.

The association of these SNPs with other clinical manifestations of AS, especially coxofemoral joint involvement and uveitis, was also evaluated. Patients with coxofemoral joint involvement or uveitis had significantly higher mSASSS scores than patients without these disease features. However, the genotype distributions and allele frequencies of these SNPs were not different. In patients with and without enthesitis, neither mSASSS nor genotype distributions nor allele frequencies of these 4 SNPs were significantly different as well.

As severe radiographic AS patients had higher acute phase reactants (patients with elevated CRP (>10mg/L) also had higher mSASSS scores, median (IQR) 0 [0-40] *vs* 0 [0-0], P=0.001) and these SNPs were supposed to have a role in inflammatory pathways, genotype distributions and allele frequencies of *ERAP1*, *IL23R* and *PTGER4* SNPs were also compared in patients with and without elevated CRP, though no difference was identified.

Clinical Determinants of Radiographic Severity

In univariate analysis, male gender (OR 4.29, P<0.001), coxofemoral joint involvement (OR 2.65, P=0.002), uveitis (OR 2.73, P=0.005) and HLA-B27 positivity (OR 2.21, P=0.013) were significantly associated with radiographic severity of AS (Table **1**). In multi-variate analysis, male gender (OR=5.95, 95% CI (2.74 to 12.90), P<0.001) and coxofemoral joint involvement (OR=2.21, 95% CI (1.14 to 4.27), P=0.018) were the sole clinical features independently associated with severe radiographic AS.

DISCUSSION

Radiographic damage in AS is a slow process, and its extent and rate varies among patients [22]. However, it is currently unknown which patients are more likely to develop significant radiographic damage of the spine and which patients are not. This is the first study to examine the association of inflammation (*ERAP1* and *IL23R*) and new bone formation (*PTGER4*) gene variants with radiographic severity of AS. We found that the risk allele frequencies of

ERAP1 rs27044 and rs30187, *IL23R* rs11209032 and *PTGER4* rs10440635 SNPs were high in AS patients but not associated with the spinal radiographic severity. Male gender and coxofemoral joint involvement were significant clinical determinants associated with severe radiographic AS.

The association between *ERAP1* rs27044 and rs30187, *IL23R* rs11209032 and *PTGER4* rs10440635 SNPs and susceptibility to AS has been previously reported in numerous studies [7, 8, 15 - 17, 23]. On the other hand, a few studies with different radiographic severity definitions addressed the association of these SNPs with radiographic severity of AS [17 - 19]. A Taiwanese study of AS patients (HLA-B27 positivity ~92%) categorized as syndesmophyte (+) and (-) reported that *ERAP1* rs27044 G allele (OR=1.59) and rs30187 T allele (OR=1.63) were modestly associated with syndesmophyte formation [19]. Another study conducted in Chinese Han AS patients showed that *PTGER4* rs10440635 AA genotype was more frequent in severe patients compared to healthy controls (severe AS definition: surgery requirement within the first 10 years of the disease due to inability to stand upright, look straight ahead, or compression of the viscera due to kyphosis) [17]. However, genotype or allele frequencies of severe and normal type of AS were not compared in that study [17]. Differences in ethnicities and radiographic severity definitions might explain the discrepancy in our study. In the Taiwanese study, the mean mSASSS of the syndesmophyte (+) and (-) groups were not reported, and the normal group patients in the Han Chinese study may also have syndesmophytes as implied by the mSASSS scores of groups (severe *vs.* normal: 36.4±20.7 *vs.* 7.71±1.86) [17, 19]. A recent study investigating the association of SNPs in genes involved in antigen presentation with radiographic severity and progression reported that *ERAP1* rs30187 SNP was associated with the baseline mSASSS in univariate analysis [18]. However, none of the examined SNPs of *ERAP1*gene were associated with radiographic progression (increase of mSASSS ≥1 unit per year) [18]. Although this is indirectly consistent with our findings, due to the slowly progressive nature of the disease, studies with longer follow-up would be more informative for differentiation of progressors and non-progressors [18]. For the *IL23R* polymorphisms, a French study reported an association between *IL23R* rs11209026 SNP and radiographic sacroiliitis in AS patients without addressing vertebral changes [24], whereas a Portugese study found no association between *IL23R* SNPs and functional status [14].

Consistent with previous reports on radiographic severity [25 - 27], we found that male gender and coxofemoral joint involvement were associated with more severe axial disease. In contrast to our previous data [28], we could not demonstrate any association between HLA-B27 positivity and radiographic severity in this cohort which might be due to the differences in severity categorization. In the prospective OASIS cohort, HLA-B27 positive male AS patients had more severe radiographic progression [29]. On the other hand, HLA-B27 was not associated with radiographic severity in the TASC and SPARCC cohorts in which the HLA-B27 negativity was low [30]. As previously reported by us and others from Turkey, HLA-B27 positivity in Turkish AS patients is relatively low (~70%) in comparison to other Caucasian populations [28, 31]. Patient selection criteria for radiographic severity and the prevalance of HLA-B27 in the study population may have implications on the associations found.

Lastly, there are several hypotheses that ankylosis starts from entheseal inflammation sites [32, 33]. However, in examination of mSASSS scores and *ERAP*, *IL23R* and *PTGER4* SNPs' genotypes of patient who had enthesitis and who did not, we could not observe any significant difference.

Clear differentiation of mild and severe radiographic AS groups is an important stratification to study the effect of genes on axial new bone formation in a disease where the rate of progression is not uniform among patients. This differentiation represents the two extremes of the radiographic severity of AS incorporating time into the equation, therefore enhancing the possibility of determining the factors that may have impact on new bone formation. Thus, the present study is the first to evaluate the effect of these SNPs on axial radiographic severity of AS in such a well-separated group of AS patients.

This study has also some limitations worth noting. First, the sample size was relatively small. However, when we compared the genotype distributions of our entire cohorts, and mild and severe groups with another Turkish AS cohort (HLA-B27 [+] 66.7%) evaluating the association of *ERAP1* polymorphisms with AS susceptibility, we found that *ERAP1* rs27044 and rs30187 genotype distributions were quite similar to ours [15]. Second, patients in the mild group had relatively shorter disease duration (mean 14.1±4.8 years) than in the severe group and it may be assumed that some of the patients in the mild group could potentially progress to the severe group over time. However, the only long-term study addressing the natural disease course of AS reported that only 26% of the AS patients with mild functional loss after ten years of disease duration experienced heavy functional loss in the follow-up [22]. Likewise, 81% of the patients who had severe spinal restriction after 36 years of follow-up were already severely restricted within the first 10 years, suggesting that a predictable pattern of ankylosing spondylitis emerges within the first 10 years of the disease.

Moreover, one of the most important predictors of future vertebral damage is prevalent syndesmophytes at baseline [3, 18, 34]. Accordingly, progression from the mild to the severe group in the next 10 years seems unlikely in our mild-AS patients who had no syndesmophytes within the first 10 years of the disease. Lastly, some of the medications may have an influence on the radiographic severity of AS. However, within the current therapeutic options only long-term NSAIDs or TNFi have been shown to retard radiographic progression to some extent [35 - 38]. Although we could not retrieve the total NSAID duration and pattern (continous or on-demand) data, previous and current NSAID exposure in both groups were not different. Additionally, TNFi use was significantly less frequent in the mild group that selection bias due to medication effect is unlikely.

Radiographic severity is a marker of long-term damage in patients with AS. Current concepts consider new bone formation as a pathological response to inflammation-mediated injury. However, there are controversial data either supporting or rebuting this hypothesis [1, 2, 39, 40]. Although patients in the severe group had a higher inflammatory burden evident with the higher CRP and TNFi use, we could not find any association between three important SNPs in genes (*ERA1P* and *IL23R*) associated with inflammation, and one SNP in *PTGER4* gene associated with new bone formation. Further studies are required to clarify the genetic determinants of radiographic progression in AS, and the functionality of these genetic variants.

CONCLUSION

This study has demonstrated that radiographic severity of AS that is substantially genetically-determined, is not associated with *ERAP1*, *IL23R* and *PTGER4* gene variants or HLA-B27.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Marmara University local ethics committee (B.30.2.MAR.0.01.02/AEK/196 Date: 23.02.2012).

HUMAN AND ANIMAL RIGHTS

No Animals were used in this research. The study involved blood samples from human subjects after obtaining written informed consent. It was conducted in compliance with the principles laid in declaration of Helsinki in 1975 and revised in 1983.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This study is supported by Marmara University, Scientific Research Projects Committee.

REFERENCES

[1] Lories RJ, Dougados M. Inflammation and ankylosis: still an enigmatic relationship in spondyloarthritis. Ann Rheum Dis 2012; 71(3): 317-8. [http://dx.doi.org/10.1136/annrheumdis-2011-200842] [PMID: 22315213]

[2] Maksymowych WP, Elewaut D, Schett G. Motion for debate: the development of ankylosis in ankylosing spondylitis is largely dependent on inflammation. Arthritis Rheum 2012; 64(6): 1713-9. [http://dx.doi.org/10.1002/art.34442] [PMID: 22354725]

[3] Baraliakos X, Listing J, von der Recke A, Braun J. The natural course of radiographic progression in ankylosing spondylitisevidence for major individual variations in a large proportion of patients. J Rheumatol 2009; 36(5): 997-1002. [http://dx.doi.org/10.3899/jrheum.080871] [PMID: 19332632]

[4] Baraliakos X, Listing J, von der Recke A, Braun J. The natural course of radiographic progression in ankylosing spondylitis: differences between genders and appearance of characteristic radiographic features. Curr Rheumatol Rep 2011; 13(5): 383-7. [http://dx.doi.org/10.1007/s11926-011-0192-8] [PMID: 21706179]

[5] Hamersma J, Cardon LR, Bradbury L, *et al.* Is disease severity in ankylosing spondylitis genetically determined? Arthritis Rheum 2001; 44(6): 1396-400. [http://dx.doi.org/10.1002/1529-0131(200106)44:6<1396::AID-ART233>3.0.CO;2-A] [PMID: 11407700]

[6] Burton PR, Clayton DG, Cardon LR, *et al.* Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat Genet 2007; 39(11): 1329-37.
[http://dx.doi.org/10.1038/ng.2007.17] [PMID: 17952073]

[7] Reveille JD, Sims AM, Danoy P, *et al.* Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. Nat Genet 2010; 42(2): 123-7.
[http://dx.doi.org/10.1038/ng.513] [PMID: 20062062]

[8] Evans DM, Spencer CC, Pointon JJ, *et al.* Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. Nat Genet 2011; 43(8): 761-7.
[http://dx.doi.org/10.1038/ng.873] [PMID: 21743469]

[9] Cui X, Hawari F, Alsaaty S, *et al.* Identification of ARTS-1 as a novel TNFR1-binding protein that promotes TNFR1 ectodomain shedding. J Clin Invest 2002; 110(4): 515-26.
[http://dx.doi.org/10.1172/JCI0213847] [PMID: 12189246]

[10] Saric T, Chang SC, Hattori A, *et al.* An IFN-gamma-induced aminopeptidase in the ER, ERAP1, trims precursors to MHC class I-presented peptides. Nat Immunol 2002; 3(12): 1169-76.
[http://dx.doi.org/10.1038/ni859] [PMID: 12436109]

[11] Murphy CA, Langrish CL, Chen Y, *et al.* Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J Exp Med 2003; 198(12): 1951-7.
[http://dx.doi.org/10.1084/jem.20030896] [PMID: 14662908]

[12] Reeves E, Elliott T, James E, Edwards CJ. ERAP1 in the pathogenesis of ankylosing spondylitis. Immunol Res 2014; 60(2-3): 257-69.
[http://dx.doi.org/10.1007/s12026-014-8576-2] [PMID: 25434650]

[13] Xin X, Majumder M, Girish GV, Mohindra V, Maruyama T, Lala PK. Targeting COX-2 and EP4 to control tumor growth, angiogenesis, lymphangiogenesis and metastasis to the lungs and lymph nodes in a breast cancer model. Lab Invest 2012; 92(8): 1115-28.
[http://dx.doi.org/10.1038/labinvest.2012.90] [PMID: 22641101]

[14] Pimentel-Santos FM, Ligeiro D, Matos M, *et al.* Association of IL23R and ERAP1 genes with ankylosing spondylitis in a Portuguese population. Clin Exp Rheumatol 2009; 27(5): 800-6.
[PMID: 19917163]

[15] Cinar M, Akar H, Yilmaz S, *et al.* A polymorphism in ERAP1 is associated with susceptibility to ankylosing spondylitis in a Turkish population. Rheumatol Int 2013; 33(11): 2851-8.
[http://dx.doi.org/10.1007/s00296-013-2824-y] [PMID: 23864143]

[16] Dong H, Li Q, Zhang Y, Tan W, Jiang Z. IL23R gene confers susceptibility to ankylosing spondylitis concomitant with uveitis in a Han Chinese population. PLoS One 2013; 8(6): e67505.
[http://dx.doi.org/10.1371/journal.pone.0067505] [PMID: 23840727]

[17] Chai W, Lian Z, Chen C, Liu J, Shi LL, Wang Y. JARID1A, JMY, and PTGER4 polymorphisms are related to ankylosing spondylitis in Chinese Han patients: a case-control study. PLoS One 2013; 8(9): e74794.
[http://dx.doi.org/10.1371/journal.pone.0074794] [PMID: 24069348]

[18] Haroon N, Maksymowych WP, Rahman P, Tsui FW, OShea FD, Inman RD. Radiographic severity of ankylosing spondylitis is associated with polymorphism of the large multifunctional peptidase 2 gene in the Spondyloarthritis Research Consortium of Canada cohort. Arthritis Rheum 2012; 64(4): 1119-26.
[http://dx.doi.org/10.1002/art.33430] [PMID: 22034108]

[19] Wang CM, Ho HH, Chang SW, *et al.* ERAP1 genetic variations associated with HLA-B27 interaction and disease severity of syndesmophytes formation in Taiwanese ankylosing spondylitis. Arthritis Res Ther 2012; 14(3): R125.
[http://dx.doi.org/10.1186/ar3855] [PMID: 22632381]

[20] van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984; 27(4): 361-8.
[http://dx.doi.org/10.1002/art.1780270401] [PMID: 6231933]

[21] Creemers MC, Franssen MJ, vant Hof MA, Gribnau FW, van de Putte LB, van Riel PL. Assessment of outcome in ankylosing spondylitis: an extended radiographic scoring system. Ann Rheum Dis 2005; 64(1): 127-9.
[http://dx.doi.org/10.1136/ard.2004.020503] [PMID: 15051621]

[22] Carette S, Graham D, Little H, Rubenstein J, Rosen P. The natural disease course of ankylosing spondylitis. Arthritis Rheum 1983; 26(2): 186-90.
[http://dx.doi.org/10.1002/art.1780260210] [PMID: 6600615]

[23] Cai G, Xin L, Wang L, *et al.* Associations between ERAP1 polymorphisms and ankylosing spondylitis susceptibility: An updated meta-analysis. Mod Rheumatol 2014; 17: 1-9.
[PMID: 25401226]

[24] Kadi A, Costantino F, Izac B, *et al.* Brief report: the IL23R nonsynonymous polymorphism rs11209026 is associated with radiographic sacroiliitis in spondyloarthritis. Arthritis Rheum 2013; 65(10): 2655-60.
[PMID: 23818276]

[25] Lee W, Reveille JD, Davis JC Jr, Learch TJ, Ward MM, Weisman MH. Are there gender differences in severity of ankylosing spondylitis? Results from the PSOAS cohort. Ann Rheum Dis 2007; 66(5): 633-8.
[http://dx.doi.org/10.1136/ard.2006.060293] [PMID: 17127685]

[26] Chen HA, Chen CH, Liao HT, *et al.* Factors associated with radiographic spinal involvement and hip involvement in ankylosing spondylitis. Semin Arthritis Rheum 2011; 40(6): 552-8.
[http://dx.doi.org/10.1016/j.semarthrit.2010.07.008] [PMID: 20870274]

[27] Boonen A, vander Cruyssen B, de Vlam K, *et al.* Spinal radiographic changes in ankylosing spondylitis: association with clinical characteristics and functional outcome. J Rheumatol 2009; 36(6): 1249-55.
[http://dx.doi.org/10.3899/jrheum.080831] [PMID: 19447933]

[28] Atagunduz P, Aydin SZ, Bahadir C, Erer B, Direskeneli H. Determinants of early radiographic progression in ankylosing spondylitis. J Rheumatol 2010; 37(11): 2356-61.
[http://dx.doi.org/10.3899/jrheum.100094] [PMID: 20843901]

[29] Ramiro S, Stolwijk C, van Tubergen A, *et al.* Evolution of radiographic damage in ankylosing spondylitis: a 12 year prospective follow-up of the OASIS study. Ann Rheum Dis 2015; 74(1): 52-9.
[http://dx.doi.org/10.1136/annrheumdis-2013-204055] [PMID: 23956249]

[30] Cortes A, Maksymowych WP, Wordsworth BP, *et al.* Association study of genes related to bone formation and resorption and the extent of radiographic change in ankylosing spondylitis. Ann Rheum Dis 2015; 74(7): 1387-93.
[http://dx.doi.org/10.1136/annrheumdis-2013-204835] [PMID: 24651623]

[31] Gunal EK, Sarvan FO, Kamali S, *et al.* Low frequency of HLA-B27 in ankylosing spondylitis patients from Turkey. Joint Bone Spine 2008; 75: 299-302.

[32] Lories RJ, Matthys P, de Vlam K, Derese I, Luyten FP. Ankylosing enthesitis, dactylitis, and onychoperiostitis in male DBA/1 mice: a model of psoriatic arthritis. Ann Rheum Dis 2004; 63(5): 595-8.
[http://dx.doi.org/10.1136/ard.2003.013599] [PMID: 15082495]

[33] Benjamin M, McGonagle D. The enthesis organ concept and its relevance to the spondyloarthropathies. Adv Exp Med Biol 2009; 649: 57-70.
[http://dx.doi.org/10.1007/978-1-4419-0298-6_4] [PMID: 19731620]

[34] Calin A, Mackay K, Santos H, Brophy S. A new dimension to outcome: application of the Bath Ankylosing Spondylitis Radiology Index. J Rheumatol 1999; 26(4): 988-92.
[PMID: 10229434]

[35] Baraliakos X, Haibel H, Listing J, Sieper J, Braun J. Continuous long-term anti-TNF therapy does not lead to an increase in the rate of new bone formation over 8 years in patients with ankylosing spondylitis. Ann Rheum Dis 2014; 73(4): 710-5.
[http://dx.doi.org/10.1136/annrheumdis-2012-202698] [PMID: 23505240]

[36] Haroon N, Inman RD, Learch TJ, *et al.* The impact of tumor necrosis factor α inhibitors on radiographic progression in ankylosing spondylitis. Arthritis Rheum 2013; 65(10): 2645-54.
[PMID: 23818109]

[37] Kroon F, Landewé R, Dougados M, van der Heijde D. Continuous NSAID use reverts the effects of inflammation on radiographic progression in patients with ankylosing spondylitis. Ann Rheum Dis 2012; 71(10): 1623-9.
[http://dx.doi.org/10.1136/annrheumdis-2012-201370] [PMID: 22532639]

[38] Wanders A, Heijde Dv, Landewé R, *et al.* Nonsteroidal antiinflammatory drugs reduce radiographic progression in patients with ankylosing spondylitis: a randomized clinical trial. Arthritis Rheum 2005; 52(6): 1756-65.
[http://dx.doi.org/10.1002/art.21054] [PMID: 15934081]

[39] Baraliakos X, Listing J, Rudwaleit M, Sieper J, Braun J. The relationship between inflammation and new bone formation in patients with ankylosing spondylitis. Arthritis Res Ther 2008; 10(5): R104.
[http://dx.doi.org/10.1186/ar2496] [PMID: 18761747]

[40] Ramiro S, van der Heijde D, van Tubergen A, *et al.* Higher disease activity leads to more structural damage in the spine in ankylosing spondylitis: 12-year longitudinal data from the OASIS cohort. Ann Rheum Dis 2014; 73: 1455-61.