

Association between single nucleotide polymorphisms in prospective genes and susceptibility to ankylosing spondylitis and inflammatory bowel disease in a single centre in Turkey

BOWEL

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ABSTRACT

Background/Aims: To establish the prevalence of the single nucleotide polymorphisms (SNPs) of endoplasmic reticulum aminopeptidase 1 (ERAP1), IL-23 receptor (IL-23R), signal transducer and activator of transcription 3 (STAT-3) and Janus kinase 2 (JAK-2) in ankylosing spondylitis (AS) and inflammatory bowel disease (IBD) in a Turkish population.

Materials and Methods: A total of 562 subjects who presented at the Ankara University internal medicine departments of rheumatology and gastroenterology outpatient clinics were recruited in this study, including 365 patients with AS, 197 patients with IBD and 230 healthy controls. ERAP1, IL-23R, STAT-3 and JAK-2) were genotyped in competitive allele-specific polymerase chain reactions.

Results: The ERAP1 (rs26653) polymorphism was found to increase the disease risk in patients with AS and IBD compared with the control group ($p=0.02$ and $p=0.01$, respectively). In addition, this polymorphism revealed a significant relationship with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and the Bath AS Functional Index (BASFI) in patients with AS ($r=0.829$, $p<0.001$ and $r=0.731$, $p<0.001$, respectively).

Conclusion: The ERAP1 gene polymorphism might be a risk factor in the pathogenesis of AS and IBD. In contrast, IL-23R gene polymorphisms may serve a protective role in AS and IBD.

Keywords: Ankylosing spondylitis, inflammatory bowel disease, single nucleotide polymorphism

INTRODUCTION

Ankylosing spondylitis (AS) is a recurring inflammatory disease that targets the spine and the peripheral joints. The prevalence of AS depends on genetic, environmental and geographical factors. Genetics accounts to 90% of the total risk for developing AS. HLA-B27 contributes to 30%–40% of the heritability of the disease. This suggests that other genetic factors also contribute to the risk for developing AS in addition to environmental factors (1,2). In addition to HLA-B27, other major histocompatibility complex (MHC) genes are known to contribute to the development of AS. 80–98% of patients

with AS exhibit positive HLA-B27, but only 5% of them develop the disease (3–5).

Inflammatory bowel disease (IBD) includes two main clinically and pathologically distinct disorders known as ulcerative colitis (UC) and Crohn's disease (CD). The etiology of IBD remains vague, and it is hypothesized that interactions among multiple factors, including genetic factors, host immune modulatory system, and environmental factors, cause a disruption of the intestinal homeostasis, leading to deregulated inflammation in the gut. Recent studies on the genetic causes of IBD have

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revealed that different immunological pathways are critical in the pathogenesis of this disorder (6).

Associations between IBD and AS have been previously identified. Approximately 6.5% of patients with AS develop IBD, and conversely, AS frequently develops in patients who are primarily diagnosed with IBD (7). In a study published in 1995, over 60% of patients with AS revealed signs of intestinal inflammation, with no major symptom of gastrointestinal distress (8). Moreover, 60–70% of patients with spondyloarthritis (SpA) were diagnosed with mainly chronic gut inflammation using colonoscopy; 7% of these patients typically developed IBD (8).

It is well known that genetic factors other than the MHC cause AS susceptibility. Recent data from two large-scale, genome-wide association studies (GWAS) revealed novel genes responsible for AS susceptibility other than HLA-B27 (9). Endoplasmic reticulum aminopeptidase 1 (ERAP1) has two central functions in the regulation of immune and inflammatory processes: (i) it cleaves the N-terminus peptides for antigen presentation by MHC-1 molecules on the endoplasmic reticulum and (ii) it trims the cytokine receptors expressed on the cell surface. Therefore, ERAP1 is an attractive candidate gene in AS. IL-23 receptor (IL-23R) is critical in the differentiation of CD4 T-cell into the Th17 lymphocyte subgroup. In several animal models of autoimmunity, the IL-23R is known to be associated with inflammation. It has been reported that Janus kinase 2 (JAK-2) and signal transducer and activator of transcription 3 (STAT-3) axes are critical for Th1 and Th17 cell differentiation, which suggests that these proteins play an important role in immune responses (10). Recent studies have demonstrated an association between the rs10758669 single nucleotide polymorphisms (SNPs) in JAK-2 and the rs744166, rs12948909 and rs229315216 SNPs in STAT-3 with IBD susceptibility (11–15). Furthermore, the rs10119004 and rs7857730 SNPs in JAK-2 and the rs6503695 SNP in STAT-3 were shown to play an indirect role in AS susceptibility in a Han Chinese cohort (13).

The effects of genetic factors in disease development may vary among ethnic groups. Hence, identification of genetic variations may be essential in AS or IBD pathogenesis. One aim of this study was to establish whether the prevalence of ERAP1, IL-23R, STAT-3 and JAK-2 gene polymorphisms in AS or IBD is high in the Turkish population compared with that observed in healthy population. Another aim of this study was to investigate the role of the indicated genes in the AS clinical manifestation patterns, including the age of symptom onset, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath AS Functional Index (BASFI) and Bath AS Metrology Index (BASMI) (6–7).

MATERIALS AND METHODS

This study was performed in patients admitted to internal medicine departments of rheumatology and gastroenterology outpatient clinics at our institution between June 2011 and July

2012. A total of 562 patients, including 365 patients with AS, 197 with IBD [78 (39.6%) with UC and 119 (60.4%) with CD] and 230 healthy matched controls were enrolled. A total of 197 patients with established IBD diagnosis were followed up by physicians at an outpatient IBD clinic in the gastroenterology department at the university hospital and were asked to participate in this study. Patient and control groups were of Turkish Caucasian ancestry. The study was approved by the local ethics committee of our institution, and we obtained written informed consent for DNA studies from patients. Hospital blood bank donors were recruited as healthy control subjects. All the patients in the case and control groups were recruited consecutively.

The necessary sample size required for this study was calculated using the MaCorr Research Toolkit, under the assumption that the ratio of risk alleles would be 60% in the test group and 40% in the control group. The required sample size to yield errors of 5% alpha and 20% beta was calculated as 165 per group. The final number of subjects with AS was 365, those with IBD was 197, and healthy subjects was 230.

Assessment

First, each patient was examined by a competent rheumatologist using standardized tests. Demographic and clinical characteristics of patients were recorded. These included age, sex, family history, age at disease onset, disease duration, history of arthritis and presence of enthesitis and uveitis. Laboratory parameters erythrocytes sedimentation rate (ESR; mm/h) and C-reactive protein (CRP; mg/L) levels were recorded. We assessed the disease status of patients by using validated measures of disease activity and function such as BASDAI, BASFI, BASMI, and the ankylosing spondylitis disease recorded activity score (ASDAS).

Genotypic analysis

Genomic DNA was obtained from whole blood samples using the Genomic DNA Purification Kit (Fermentas; Vilnius, Lithuania). Five candidate genes and SNPs, including IL-23R (rs11209032), JAK-2 (rs10974944), STAT-3 (rs8074524) and ERAP1 (rs26653), were genotyped in patient samples, as previously described (Sato, Trifa, DINA MAREK-YAGEL, Zhou) (11). HLA-B27 typing was performed using the PCR-SSP technique with a commercial HLA-B*27 kit (Olerup SSP).

The primers used for PCR-restriction fragment-length polymorphism (RFLP)-based assays are as follows: IL-23R: forward-5'-CTCCCTACATCACCTCTTTGCACT-3' and reverse-5'-TGATAAGGCAATCCGGTGGTTC-3'; JAK-2: forward-5'-CAAGGGTCAACTGTAGTACATAA-3' and reverse-5'-CTGCTTGC-TAGTGGGTGAAT-3'; STAT-3: forward-5'-GTCTGGAAAGCTCCATCTGC-3' and reverse-5'-AGAGGCCAGATTAGTGCTGG-3'; ERAP: forward-5'-CGTGGAAAAGTCTCCGAAAGA-3' and reverse-5'-TCCTG-CATAGTACCACCTG-3'.

PCR reactions were performed in 25- μ L reaction vessels containing 2 μ L DNA, 1 \times buffer (MBI Fermentas; Vilnius, Lithuania),

0.2 mM of each dNTP, 0.4 µM each of forward and reverse primers, 2 mM MgCl₂, and 1 U Taq DNA Polymerase (MBI Fermentas; Vilnius, Lithuania). Amplification was performed in a GeneAmp PCR System 9700 instrument (PE Applied Biosystems; Foster City, CA, USA) and the cycling conditions were as follows: denaturation at 95°C for 2 min, 35 cycles each of denaturation at 95°C for 50 s; annealing at 60°C (57°C for STAT-3 and JAK-2) for 50 s; extension at 72°C for 50 s and final extension at 72°C for 5 min. Amplified PCR products were digested with Bfal (New England Biolabs, Inc.; Ontario, Canada) for the IL-23R SNP; Mbol (MBI Fermentas; Vilnius, Lithuania) for the JAK-2 SNP, HpaII (MBI Fermentas; Vilnius, Lithuania) for the STAT-3 SNP and Ddel (New England Biolabs Inc.; Ontario, Canada) for the ERAP-1 SNP. Products were segregated on a 2% agarose gel and stained with 10 µL ethidium bromide.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 15.0 (SPSS Inc.; Chicago, IL, USA). Descriptive statistics are given as mean±standard deviation and frequency. The normality of distribution was tested using one-sample Kolmogorov–Smirnov test. Consecutive variables between groups were analysed using the Student’s t-test and Mann–Whitney U test. One-way analysis of variance (ANOVA) and Kruskal–Wallis tests were used to compare the differences among groups.

We compared categorical data using Pearson’s Chi-squared test. The accepted level of significance was 5%. However, when we estimated the significance of more than 2 SNPs simultaneously, the threshold used for statistical significance was set at a p value of 0.05 divided by the number of SNPs compared. The genotyping certainty of SNPs in patients and controls was confirmed using the Hardy–Weinberg Equilibrium (HWE). The validity of the HWE was confirmed using the Chi-squared test. The Pearson’s Chi squared test was used to analyse variations in genotype and allele rates. Results were expressed as odd ratios (ORs) with a 95% confidence interval. Age and HLA-B27 adjusted odds ratios for candidate SNPs were computed with dual logistic regression analysis while the phenotypic expressions among groups were analysed with Chi-squared tests.

RESULTS

365 patients with AS, 197 patients with IBD, and 230 healthy controls were included in the study. The mean age of subjects with AS and IBD was 38.5±11.6 and 40.1±12.2 years, respectively, and the average disease duration was 7.3±6.2 and 6.1±5.7 years, respectively. The mean age of the control group was 34.1±9.6 years. Positive HLA-B27 rate was 80%, 20.3%, and 7.8% in the AS, IBD and control groups. Characteristics of patients and control group subjects are shown in Table 1 and Figure 1. Genetic analyses revealed that ERAP1, STAT-3, IL-23R and JAK-2 were in HWE in the AS, IBD [including CD and UC] and control groups.

Table 1. Demographic and clinical characteristics of patients with AS or IBD and controls

Characteristics	AS (n=365)	IBD (n=197)	Control (n=230)
Mean±SD or n (%)			
Age, years	38.5±11.6	40.1±12.2	34.1±9.6
Sex			
Female	113 (31)	102 (51.8)	114 (49.6)
Male	252 (69)	95 (48.2)	116 (50.4)
HLA-B27 positivity	292 (80.0)	40 (20.3)	18 (7.8)
Disease duration, years	7.3±6.2	6.1±5.7	-
Positive Family History	158 (43.3)	44 (22.3)	-
Current smoker	139 (38.1)	47 (23.9)	-
Anterior Uveitis	77 (21.1)	7 (3.6)	-
Enthesitis	145 (39.7)	68 (34.5)	-
Peripheral arthritis	62 (17)	33 (16.8)	-
BASDAI	6.3±1.4	-	-
BASMI	2.6±2.3	-	-
ASDAS CRP	3.3±0.7	-	-
ESR (mm/h)	35±26	39±27	-
CRP (mg/L)	24±53	27±37	-

AS: ankylosing spondylitis; ASDAS: ankylosing spondylitis disease activity score; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASMI: Bath Ankylosing Spondylitis Metrology Index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IBD: inflammatory bowel disease

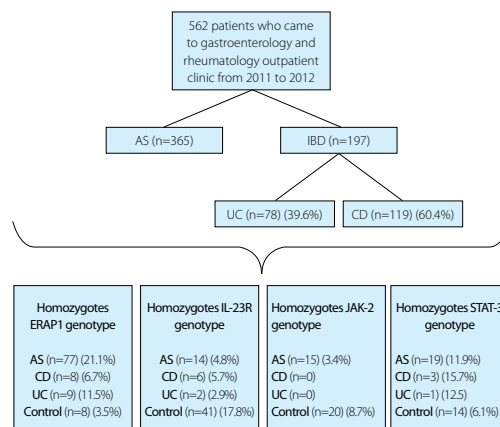


Figure 1. Flowchart showing patient groups and their genotypes

rs26653 SNP C/C homozygotes were detected in 77 (21.1%) patients with AS, 8 (6.7%) patients with CD, 9 (11.5%) patients with UC and 8 control patients (3.5%). The frequency pattern of rs26653 SNP C/C homozygous genotype in AS patients was statistically significant (p=0.024; Table 2). The frequency of rs26653 risk allele was higher in AS (44%) compared with controls (23%; OR: 2.81; p<0.001). We also detected rs26653 SNP C/C homozygotes in 17 IBD patients and 8 controls (3.5%). This frequency distribution of rs26653 SNP C/S was statistically sig-

Table 2. SNP allele frequency distributions among AS patients and controls

Gene (SNPs)	Allele frequency, n (%)			Genotype distribution, n (%)				
		AS	Control		AS	Control	p	OR (95% CI)
ERAP1 (rs26653)	G	411 (56)	355 (77)	GG	120 (32.9)	133 (57.8)	0.01	0.51 (0.35–0.76)
	C	319 (44)	105 (23)	GC	168 (46.0)	89 (38.7)	0.02	1.56 (1.06–2.3)
				CC	77 (21.1)	8 (3.5)	*0.02	2.62 (1.10–6.21)
IL-23R (rs10974944)	G	430 (73)	260 (56)	GG	158 (53.9)	70 (30.4)	<0.01	2.49 (1.73–3.58)
	A	156 (27)	200 (44)	GA	121 (41.3)	119 (51.7)	0.07	0.70 (0.47–1.03)
				AA	14 (4.8)	41 (17.8)	*0.01	0.23 (0.12–0.43)
STAT-3 (rs8074524)	C	217 (75)	364 (79)	CC	82 (51.2)	146 (63.5)	0.055	0.46 (0.20–1.03)
	T	103 (25)	96 (21)	CT	59 (36.9)	70 (30.4)	0.276	1.57 (0.69–3.55)
				TT	19 (11.9)	14 (6.1)	0.093	2.68 (0.81–8.83)
JAK-2 (rs10974944)	A	337 (71)	312 (67)	AA	115 (57.5)	102 (44.3)	0.147	1.30 (0.90–1.88)
	C	133 (29)	148 (33)	AC	105 (39.1)	108 (47)	0.389	0.85 (0.59–1.22)
				CC	15 (3.4)	20 (8.7)	0.147	0.76 (0.53–1.10)

*p<0.05 following Bonferroni correction were considered statistically significant.

SNPs: single-nucleotide polymorphisms; AS: ankylosing spondylitis; ERAP1: endoplasmic reticulum aminopeptidase 1; IL-23R: IL-23 receptor; STAT-3: signal transducer and activator of transcription 3; JAK-2: Janus kinase 2

nificant ($p=0.024$). The rs26653 risk allele was more frequent in patients (34%) than in controls (23%; OR: 2.66; $p<0.001$).

According to IBD subtypes, rs26653 SNP C/C homozygotes were detected in 8 (6.7%) CD patients and 8 (3.5%) controls ($p=0.19$). rs26653 SNP C/C homozygotes were detected in 9 (11.5%) UC patients and 8 (3.5%) controls. The difference in frequencies of rs26653 SNP C/C was statistically significant in both groups ($p=0.03$). The frequency of the rs26653 risk allele was higher in patients with CD (41%) and UC (34%) than in control subjects (23%), as shown in Table 3.

We detected IL-23R (rs11209032) SNP A/A homozygotes in 14 AS patients (4.8%) and 41 control subjects (17.8%). This variation in the frequency distribution was statistically significant ($p<0.01$), as shown in Table 2. The frequency of the rs11209032 risk allele was higher in controls (44%) than in AS patients (27%; OR: 0.39; $p<0.001$).

We detected IL-23R (rs11209032) SNP A/A homozygotes in 8 (4.6%) IBD patients and 41 (17.8%) controls. This variation in the frequency distribution was also statistically significant ($p<0.01$). The frequency of the rs11209032 risk allele was higher in controls (44%) than in IBD patients (26%; OR: 0.40; $p<0.001$). Similarly, the variation in the frequency distribution of SNP A/A homozygotes genotype in patients with CD and UC was statistically significant (both $p=0.01$). The frequency of the rs11209032 risk allele was higher in controls (Table 3).

We detected STAT-3 (rs8074524) SNP T/T homozygotes in 19 (11.9%) AS patients and 14 (6.1%) controls. This frequency distribution difference of the STAT-3 (rs8074524) SNP T/T homozygotes genotype was not observed to be statistically significant

($p=0.093$). The frequency of the rs11209032 risk allele was not found to be statistically significant (OR 2.25; $p=0.054$).

We found JAK-2 (rs10974944) SNP G/G homozygotes in 15 (3.4%) AS patients and 20 (8.7%) controls. This frequency distribution difference of the JAK-2 (rs10974944) SNP G/G homozygote genotype was not observed to be statistically significant ($p=0.147$). The frequency of the rs10974944 risk allele was not found to be statistically significant (OR: 0.76; $p=0.147$).

We also found a significant association between the incidence of the ERAP1 (rs26653) SNP and HLA-B27 positivity rate ($p<0.001$). The frequency distributions of several clinical (presence of uveitis, peripheral arthritis or anterior uveitis) and demographic (sex, family history of SpA, age at disease onset) features were similar for patients who had different rs26653 SNP genotypes (C/C, C/S or G/G), except for individuals with enthesitis in whom the rs26653 C/C SNP genotype was observed more frequently than in those without enthesitis. Similarly, the mean BASDAI, BASFI, BASMI and ASDASCRP values were higher in patients with the rs26653 C/C SNP genotype than in other patients. These differences were statistically significant ($p=0.01$, $p=0.027$, $p<0.001$, and $p=0.010$, respectively). Multivariate logistic regression analysis performed for AS and healthy controls revealed significant associations between the IL-23 risk allele (A), HLA-B27 and age (Table 4).

21 (10.6%) of the 197 IBD patients fulfilled the criteria for AS. We found rs26653 SNP C/C homozygotes in 6 (28.6%) IBD patients with AS and 8 controls (3.5%). This difference in the frequency distribution of the rs26653 SNP C/C homozygous genotype between the two groups was statistically significant ($p=0.001$). We

Table 3. Distribution of SNP genotype frequencies among patients with IBD, UC, CD and controls

Gene (SNPs)	Allele frequency, n (%)					Genotype distribution, n (%)									
	IBD (n=197)	CD (n=119)	UC (n=78)	Control (n=230)		IBD	p	OR (95% CI)	CD	p	OR (95% CI)	UC	Control	p	OR (95% CI)
ERAP-1 (rs26653)	G	187 (62)	111 (59)	76 (66)	355 (77)	GG	0.01	0.51 (0.35-0.76)	43 (36.1)	0.02	0.62 (0.42-0.94)	39 (50)	133 (57.8)	0.51	0.86 (0.55-1.34)
	C	115 (88)	76 (41)	39 (34)	105 (23)	GC	0.02	1.56 (1.06-2.3)	68 (57.2)	0.04	1.47 (1.0-2.17)	30 (38.5)	89 (38.7)	0.98	0.99 (0.61-1.61)
						CC	0.00	2.66 (1.10-6.21)	8 (6.7)	0.19	1.93 (0.7-5.2)	9 (11.5)	8 (3.5)	*0.03	2.94 (1.07-8.12)
IL-23R (rs10974944)	G	167 (67)	100 (65)	67 (68)	260 (56)	GG	<0.01	2.47 (1.64-3.72)	55 (51.8)	0.01	1.70 (1.11-2.19)	39 (56.5)	70 (30.4)	0.01	1.80 (1.15-2.9)
	A	83 (33)	52 (35)	31 (32)	200 (44)	GA	0.07	0.70 (0.47-1.03)	45 (42.5)	0.34	0.82 (0.54-1.24)	28 (40.6)	119 (51.7)	0.33	0.78 (0.47-1.28)
						AA	*0.01	0.28 (0.13-0.57)	6 (5.7)	*0.01	0.31 (0.13-0.77)	2 (2.9)	41 (17.8)	*0.01	0.16 (0.03-0.68)

*p<0.05 following Bonferroni correction were considered statistically significant.

SNPs: single-nucleotide polymorphisms; AS: ankylosing spondylitis; ERAP1: endoplasmic reticulum aminopeptidase 1; IL-23R: IL23 receptor; CD: Crohn's disease; UC: ulcerative colitis; IBD: inflammatory bowel disease; OR: odds ratio; CI: confidence interval

Table 4. Multivariate logistic regression analysis between patients with AS and healthy control subjects

	β	p	OR (95% CI)
IL-23R risk allele (A)	-0.728	0.030	0.483 (0.25-0.93)
HLA-B27	3.923	<0.001	50.563 (24.6-103.8)
Age	0.065	<0.001	1.067 (1.06-1.09)

OR: odds ratio; CI: confidence interval

also found IL-23R (rs11209032) SNP A/A homozygotes in 6 (3.9%) IBD patients without AS and 41 (17.8%) controls. This difference in the frequency distribution was statistically significant (p<0.01).

DISCUSSION

To the best of our knowledge, this study is the first to investigate the relationships among ERAP1, IL-23R, STAT-3 and JAK-2 in patients with AS and IBD. In this study, we discovered a strong association between ERAP1 (rs26653) SNP and AS (OR: 2.62, p=0.02). To the best of our knowledge, this study is the first to show a significant correlation between this polymorphism and IBD (OR: 2.66; p<0.01), suggesting that the ERAP1 (rs26653) polymorphism may be an important genetic factor influencing the pathogenesis of IBD subgroups, particularly UC (OR: 2.94, p=0.03) with axial involvement, in addition to AS. Eighty percent of all patients showed positivity for HLA-B27. In a study conducted by Gunal et al. (16) evaluating demographic data of 122 subjects, the rate of HLA-B27 positivity was 70%. In another multicentre study performed by Bodur et al. (17), the HLA-B27 prevalence was 73.7% in 262 patients with AS, which is consistent with the results obtained in other studies conducted in Turkey.

Several studies that investigated the correlation between ERAP1 polymorphisms and AS have reported conflicting results (6-13). Possible factors for this discrepancy include clinical heterogeneity, genetic heterogeneity, small sample size and low statistical power.

In a large-scale study by Maksymowych et al. (18) investigating the relationship between AS and ERAP1 gene polymorphisms, a strong correlation was found between ERAP1 (rs27044, rs10050860, rs30187 and rs26653) SNPs and AS. Similarly, in a study by Kadi et al. (19) of 734 patients with AS, a significant correlation was found between ERAP1 (rs27044, rs301187 and rs1742078) SNPs and the risk for AS (OR: 1.34; p=0.005; OR: 1.31, p=0.008; and OR: 0.72, p=0.011, respectively).

In the study by Mahmoudi et al. (20), it was established that in 387 patients with AS, the ERAP1 (rs30187) SNP increased the risk for AS (OR: 2.7, p<0.001), while the ERAP1 (rs27434) SNP decreased the disease risk (OR: 0.48, p<0.005), making it the first study to demonstrate functional roles of ERAP1 SNPs in AS.

In a review by Alvarez-Navarro et al. (21), various ERAP1 SNPs in AS were discussed. In studies performed in Europe, America and the Far East, it has been reported that ERAP genes increase

Table 5. Distribution of SNP genotype frequencies among IBD patients with AS and without AS

Gene (SNPs)	Allele frequency, n (%)				Genotype distribution, n (%)					
		With AS (n=21)	Without AS (n=176)		With AS (n=21)	p	OR (95% CI)	Without AS (n=176)	p	OR (95% CI)
ERAP-1 (rs26653)	G	15 (56)	165 (61)	GG	9 (42.8)	0.46	0.74 (0.31-1.66)	73 (41.5)	0.06	0.71 (0.50-1.01)
	C	12 (44)	103 (39)	GC	6 (28.6)	0.52	0.73 (2.6-25.9)	92 (52.2)	0.09	1.35 (0.95-1.91)
				CC	6 (28.6)	*0.001	8.2 (0.7-5.2)	11 (6.2)	0.21	1.79 (0.70-4.56)
IL-23R (rs10974944)	G	19 (82)	148 (65)	GG	17 (81)	0.01	2.6 (1.11-2.19)	77 (50)	0.01	1.64 (1.12-2.40)
	A	4 (18)	79 (35)	GA	2 (9.5)	0.00	0.82 (1.32-5.31)	71 (46.1)	0.52	0.89 (0.62-1.27)
				AA	2 (9.5)	0.40	0.53 (0.12-2.36)	6 (3.9)	*0.00	0.21 (0.09-0.52)

*p<0.05 following Bonferroni correction were considered statistically significant.
SNPs: single-nucleotide polymorphisms; AS: ankylosing spondylitis; ERAP1: endoplasmic reticulum aminopeptidase 1; IL-23R: IL23 receptor; IBD: inflammatory bowel disease; OR: odds ratio; CI: confidence interval

the risk for developing the disease, particularly in cases involving HLA-B27 positivity. It was demonstrated that the ERAP1 SNPs responsible for an increased risk for developing AS are rs26653 (OR: 1.3), rs30187 (OR: 1.4) and rs27044 (OR: 1.3).

A study performed by Cinar et al. (22) was the first to address the relationship between ERAP1 and AS in a Turkish population. Unlike meta-analysis involving Caucasian races, an interaction between rs26653 and AS was demonstrated in the Turkish population. The findings of the present study are similar to those in the study by Cinar et al. (22) with respect to the relation between ERAP1 (rs26653) and AS; however, we also found a significant relation between ERAP1 and HLA-B27. ERAP1 (rs26653) may also contribute to AS pathogenesis, particularly in HLA-B27-positive patients, although this finding was not supported by the study by Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al. (9). The relation between ERAP1 (rs26653) and AS in the Turkish community was shown in two studies. A relation between the rs30187 and rs27044 SNPs and HLA-B27 was demonstrated in connection with disease, particularly in meta-analysis and GWAS studies. rs26653, which appears to be a risk allele in the Turkish population, may influence the ATP-binding reserve and transport efficacy of TAP, or alter the substrate specificity and proteolytic capability of the immunoproteasome.

In the literature, the results of some studies have demonstrated positive correlations between ERAP1 SNPs and disease activity and functional status, while the results of other studies have demonstrated no such relationship. A study by Szczypiorska et al. (23) was the first to report a correlation of ERAP1 gene SNPs with functional status in AS patients (BASFI scores, corrected for disease duration). Moreover, Wang et al. (24) detected a significant correlation between ERAP1 and the development of syndesmophytes.

The finding that JAK-2 and STAT-3 play a critical role in the differentiation of Th1 and Th17 cells indicated that these molecules

serve important functions in the development of immune responses. It is believed that JAK-2 and STAT-3 gene mutations contribute to AS pathogenesis by causing an abnormality in the Th17 cellular function (25).

Results from recent CD GWASs have broadened our understanding of the genetics of IBD (26–29). The study conducted by Duerr et al. (15) reported a strong association of IBD with the IL-23R gene. The non-synonymous rs11209026 SNP (R381Q) in the IL-23R gene was found to provide a strong shield against CD. However, results from a study of patients with AS in the United Kingdom revealed that the IL-23R (rs11209032) SNP was most strongly associated with IBD ($p < 0.001$) (30). In a Spanish study, associations with IL23-R SNPs were observed to exert a protective effect against IBD (rs1343151, $p < 0.0002$; rs11209026, $p < 0.001$; and rs10889677, $p < 0.04$). In contrast, a meta-analysis involving four different IL23-R SNPs (rs1004819, rs10489629, rs11209032 and rs1495965) did not demonstrate an association with AS (31).

In another GWAS study, it was found that STAT-3 (rs2293152) SNP was protective against the risk for developing AS (OR: 0.81; $p = 0.0016$) (32). Davidson et al. (33) demonstrated that TNFRF1A (rs4149577, $p = 0.008$) and STAT-3 (rs2293152, $p = 0.0015$; rs1053005, $p = 0.017$) SNPs had a significant correlation with AS. In the present study, as patients with AS were matched with controls, no significant relation was found between STAT-3 (rs8074524) and the risk for disease (OR: 0.60, $p = 0.242$); however, it was shown that the JAK-2 (rs10974944) vs. IL-23R (rs112209032) SNPs played protective roles by significantly decreasing the disease risk (OR: 0.37, $p = 0.0217$ vs. OR: 0.25, $p = 0.0001$, respectively). Different results obtained here compared to those in previous studies may be attributable to the diverse racial and genetic characteristics in different societies. Alternatively, mutations in the same gene may influence the risk for disease by causing varying immunologic effects.

Karaderi et al. (30) conducted a case-control and meta-analysis study investigating the relation between IL-23R and AS in 3482 patients with AS and 3150 controls. In that study, the authors found that some SNPs (rs1004819, rs11209032 and rs1495965 with ORs of 1.21, 1.24 and 1.19, respectively) were associated with AS, while other SNPs (rs10489629, rs11465804, rs11209026 and rs1343151, with ORs of 0.85, 0.62, 0.61 and 0.82, respectively) were protective against AS. In contrast, results from a study by Pimentel-Santos et al. (34) in a Portuguese population showed that the only IL-23R SNP related to AS was rs1004819 (OR: 1.4, p=0.0049). In addition, no correlation with AS was found for the IL-23R (rs11209032) SNP, which was most strongly associated with AS in a meta-analysis of British patients (30), nor was any significant correlation found with IL-23R (rs11209026), which is protective.

When AS patients were compared with controls, it was shown that IL-23R (rs112209032) SNPs played a protective role in both AS and IBD by reducing the disease risk significantly. Results from previous studies have shown that different IL-23R SNPs can either positively or negatively influence disease development. In particular, the interrelationship between IL-23 and JAK-2 may serve a protective role by decreasing cellular responses to IL-23-related signalling, which may have also caused discordant results between this study and previous studies.

Guerini et al. (35) first reported the relationship of ERAP1 with IBD, demonstrating that the ERAP1 (rs31087) AA genotype risk allele was present more commonly in patients with CD (15%) than in healthy control subjects (11%; OR: 1.20; p=0.036). Similarly, results of this study showed the ERAP1 (rs26653) allele may confer increased risk for IBD on the Turkish population studied here.

The ERAP1 homolog ERAP2 was found to be associated with IBD in a previous IBD GWAS study, which showed a strong relationship between ERAP1/2 and AS, providing evidence for a potential causal link with IBD (36).

deVlam et al. (7) reported that 10% of IBD patients fulfilled the criteria for ankylosing spondylitis. Similarly, our results showed 21 (10.6%) IBD patients with AS.

A limitation of this study is that we did not examine ERAP1, JAK-2, STAT-3 and IL-23R polymorphisms in individuals with psoriatic arthritis, who have a relatively high risk for developing SpA. Also, we studied a small number of genotypes associated with CD and UC, which are relatively common among European populations. Established gene polymorphisms related to IBD, such as those occurring in ATG16L1, NOD and CARD, could have been investigated regarding their association with the IL-1 pathway. Another limiting factor is the cross-sectional nature of the study, which incorporated modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) scores into patient activity scores while evaluating correlations between disease activity and ERAP1. Investigating disease activity on risk alleles

by the inclusion of BASDAI and ASDAS scores as well as CRP in the evaluation is merited for prospective studies.

In conclusion, IL-23R gene polymorphisms in the Turkish population may serve an important and protective role in AS and IBD by preventing disease risk, and ERAP1 polymorphism may increase the risk for developing AS and IBD. We demonstrated that ERAP1 gene polymorphisms in patients with AS were also significantly correlated with disease severity parameters, as measured by BASDAI, BASMI, ASDASCRP and BASFI evaluations. Moreover, a significant correlation was shown with HLA-B27.

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Informed Consent: Written informed consent was obtained from patients who participated in this study.

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