Association of *ARTS1* Gene Polymorphisms with Ankylosing Spondylitis in the Hungarian Population: The rs27044 Variant Is Associated with HLA-B*2705 Subtype in Hungarian Patients with Ankylosing Spondylitis

BORBÁLA PAZÁR, ENIKO SÁFRÁNY, PÉTER GERGELY, SÁNDOR SZÁNTÓ, ZOLTÁN SZEKANECZ, and GYULA POÓR

ABSTRACT. ***Objective.*** Associations have been found between ankylosing spondylitis (AS) and polymorphisms in the aminopeptidase regulator of TNFR1 shedding (ARTS1) gene. We studied the association of 5 polymorphisms within the *ARTS1* gene with AS in Hungarian patients. We also investigated the prevalence of HLA-B27 subtypes in the Hungarian population.

Methods. A case-control study including 297 patients with AS and 200 sex and ethnically matched healthy controls was performed. Patients and controls were genotyped for rs27044, rs17482078, rs10050860, rs30187, and rs2287987 single-nucleotide polymorphisms using real-time polymerase chain reaction (PCR) allelic discrimination. HLA-B27 subtypes were determined with PCR sequence-specific primer (PCR-SSP) technique.

Results. We observed a significant increase in the minor allele frequency of rs27044 ($p = 0.001$) in the AS group compared to controls. The minor allele frequencies of rs10050860 ($p = 0.006$) and rs2287987 ($p = 0.002$) showed a significant decrease in AS patients compared to controls. Haplotype analysis revealed association of 2 ARTS1 haplotypes with AS in the Hungarian population. We found that HLA-B*2705 was the predominant subtype in Hungarians with AS. Carriage of the G allele of rs27044 was significantly associated with the HLA-B*2705 subtype ($p = 0.009$) in AS patients.

Conclusion. We confirmed reported associations of *ARTS1* gene polymorphisms with AS in a Hungarian cohort study. We found HLA-B*2705 as the predominant subtype in Hungarian AS patients in accord with other studies on Caucasian populations. Our results suggest that the *ARTS1* gene variants together with HLA-B27 strongly contribute to disease susceptibility in patients with AS. (J Rheumatol First Release Dec 23 2009; doi:10.3899/jrheum.090806)

Key Indexing Terms:
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From the National Institute of Rheumatology and Physiotherapy, Budapest; Department of Medical Genetics and Child Development, University of Pécs, Pécs; and Department of Rheumatology, Institute of Medicine, University of Debrecen Medical and Health Science Center, Debrecen, Hungary.

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B. Pazár, MD, National Institute of Rheumatology and Physiotherapy; E. Sáfrány, Department of Medical Genetics and Child Development, University of Pécs; P. Gergely Jr, MD, PhD, National Institute of Rheumatology and Physiotherapy; S. Szántó, MD, PhD; Z. Szekanecz, MD, PhD, DSc, Department of Rheumatology, Institute of Medicine, University of Debrecen Medical and Health Science Center; G. Poór, MD, PhD, DSc, National Institute of Rheumatology and Physiotherapy.

Address correspondence to Dr. Z. Szekanecz, Rheumatology Division, Third Department of Medicine, University Medical School of Debrecen, 98. Nagyerdei krt., Debrecen, H-4032 Hungary.
E-mail: szekanecz.zoltan@med.unideb.hu

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Ankylosing spondylitis (AS) is an inflammatory disorder of unknown etiology characterized by spine, peripheral joint, or entheseal involvement that is variably associated with extraarticular manifestations. The disease shows increased prevalence among individuals carrying the HLA-B27 gene, although the exact pathogenic significance of this association has not been fully elucidated[1]. Studies have implicated several new non-major histocompatibility complex (MHC) genes in susceptibility to AS[2,3]. One of the most interesting findings has been the association of the *ARTS1* gene with the disease[4-6]. Aminopeptidase regulator of TNFR1 shedding (ARTS1) is an endoplasmic reticulum-associated aminopeptidase (also known as ERAP1). It has 2 known functions: it is involved in peptide trimming for class I MHC presentation[7,8] and it cleaves cell-surface receptors of

proinflammatory cytokines[9-11]. The *ARTS1* gene is located on chromosome 5q15. In the Wellcome Trust Case Control Consortium (WTCCC) AS study involving 1000 cases and 1500 controls, 2 single-nucleotide polymorphisms (SNP) on chromosome 5 reached a level of statistical significance for association with AS, both of which corresponded to areas within the *ARTS1* gene (rs27044 and rs30187). Three additional *ARTS1* SNP (rs17482078, rs10050860, rs2287987) are associated with AS at modest significance levels[2].

The *ARTS1* association has been confirmed in Caucasian[12,13] and Korean[14] populations. All 3 studies report that variants containing the *ARTS1* rs27044 or the rs30187 SNP have the strongest association with the prevalence of AS. Haplotypic analysis revealed the rs27044/10050860/30187-CCT haplotype as a susceptibility factor for AS in a Canadian cohort of patients and controls with Caucasian and northern European origin[12]. In familial AS a combined haplotype from the *ARTS1* (*ERAP1*) and *ERAP2* genes, the rs27044/30187/2549782-GTT, showed excess transmission in Caucasian AS multiplex families[13]. In a Korean population, the rs27044/17482078/ 10050860/ 30187-GCCT and the rs27044/17482078/ 10050860/ 30187-CCCC haplotypes were associated with AS[14].

The other non-MHC gene recently confirmed to be strongly, independently associated with AS is *IL23R*[5]. It affects susceptibility to Crohn's disease[15,16] and psoriasis as well[17]. The *IL23R* gene is located on chromosome 1p31 and the encoded protein forms a receptor for interleukin 23 IL-23, together with the ß1 subunit of IL-12 (IL-12Rß1)[18]. IL-23, a member of the IL-12 cytokine family, is a proinflammatory cytokine that plays a central role in the differentiation of native CD4+ T cells into IL-17-producing T helper cells[19].

In addition to *ARTS1* and *IL23R*, other non-MHC genes show association with AS. Several polymorphisms of the interleukin 1 (IL-1) gene cluster on chromosome 2q13 are implicated in susceptibility for AS in Caucasian and Asian populations[5]. A metaanalysis performed on 9 polymorphisms in the *IL1* gene cluster members showed that 3 SNP in the *IL1A* gene were associated with susceptibility to AS in a large cohort of different populations[20].

The cytochrome P450 *CYP2D6* gene on chromosome 22q13.1 is also reported to show a weak association with AS in case-control studies. Homozygosity for poor metabolizer alleles (CYP2D6*4) was found to be associated with AS but not with rheumatoid arthritis[21,22].

The strong association between HLA-B27 and AS has been known since 1973[23,24]. A series of amino acid substitutions clustered around the antigen-binding site generate the HLA-B27 allelic variants. These could influence susceptibility to AS either by altering antigenic epitopes on the HLA-B27 molecule itself, or by affecting the range of peptides that these HLA-B27 variants bind. The commonest alleles in Caucasian populations are HLA-B*2705 and -B*2702 and the Asian variant is HLA-B*2704[25,26]. For Central and Eastern Europe there is only one study describing the association of B27 subtypes is AS[27].

As major geographic and ethnic variations have been observed when various non-MHC genes were studied in association with arthritis in recent years, we investigated whether rs27044, rs30187, rs17482078, rs10050860, and rs2287987 *ARTS1* polymorphisms contribute to disease susceptibility in Hungarian patients with AS. While both *ARTS1* and HLA-B27 associations of AS have been established, the possible connection between HLA-B27 and the presence of any of the *ARTS1* variants in the context of AS remains unclear. Therefore, we also examined potential coincidence of HLA-B27 subtypes and *ARTS1* variants in Hungarian patients with AS.

MATERIALS AND METHODS

Patients and controls. DNA samples were obtained from 297 Hungarian AS patients (213 men, 84 women) with a mean age of 39.2 (± 5.6) years. AS patients were recruited from the National Institute of Rheumatology and Physiotherapy in Budapest and the Department of Rheumatology at the University of Debrecen Medical and Health Sciences Center, Debrecen, Hungary. AS was defined according to the modified New York diagnostic criteria[28]. The diagnosis of AS was established in all patients by a qualified rheumatologist. Altogether, 200 sex- and ethnically matched healthy individuals (83 men, 117 women) with a mean age of 45.2 (± 4.1) years served as controls. For the evaluation of HLA-B27 subtype data, we used a different control group of 70 HLA-B27-positive healthy individuals (37 men, 33 women, mean age 45.05 ± 17.50 yrs). Control subjects were all recruited from the National Medical Center, Institute of Haematology and Immunology, Budapest. All individuals were unrelated Hungarian Caucasians. Informed written consent was obtained from each participant, and local institutional review board approval was obtained at both recruitment sites. This study was performed according to the Declaration of Helsinki.

ARTS1 and HLA-B27 genotyping. DNA was isolated from peripheral blood samples with the Genomic DNA Purification Tray II (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions using a Nucleic Acids PreStation 6100 instrument (Applied Biosystems). DNA samples were stored at 4°C until use. The *ARTS1* SNP analysis was performed with real-time PCR allelic discrimination TaqMan assays (Applied Biosystems). Real-time PCR analysis was carried out in a total volume of 10 μl with 10 ng of genomic DNA, 1 pmol gene-specific forward and reverse primer in 1× TaqMan 2× Universal PCR Master Mix No AmpErase UNG (Applied Biosystems). Specifically, forward TaqMan primers for the ARTS1 alleles were as follows, rs27044: TGC ACA CAG GCG AGG AGT AGT AGT T[C/G]AC TCC GCA GCA TTC GCT CTG AGA CT; rs17482078: GAG TAG TAG TTG ACT CCG CAG CAT T[C/T]GC TCT GAG ACT GAG CCC TCG TCT GT; rs10050860: TTT AGC AAA AAT CGA TGG ACC ATG T[C/T]GG ATT TGC TGG TGA TGA ATG TCA AT; rs30187: TGT GAT GGT TAT TAG GGG AAA ACC C[C/T]TC TGC AGT GTC CAA GTG TTC ATC AT; rs2287987: TGA GCC AGT TCA TGG GCC ACA GTC A[C/T]TG TGA TGC CAA GCT TAC TTG ATG CA.

Real-time PCR was performed using an ABI 7300 Real-Time PCR System (Applied Biosystems) according to the manufacturer's instructions.

HLA-B27 typing was performed using the PCR sequence-specific primer (PCR-SSP) technique (Histo Type B27 high resolution kit; BAG, Lich, Germany). HLA-B27 subtypes were also determined with PCR-SSP with the Olerup SSP™ HLA*B27 kit (Olerup SSP AB, Hasselstigen, Sweden).

Statistical analysis. Allele and genotype frequencies were tested for Hardy-

Weinberg equilibrium in case and control groups. Association tests for allele frequencies of each SNP and in cases and controls were performed using the chi-square test. A Bonferroni corrected $p < 0.01$ was considered significant for multiple comparisons. Linkage disequilibrium coefficient D' and r^2 were determined using Haploview version 4.1[29]. Haplotype frequencies were estimated using PHASE version 2.1[30,31].

RESULTS

The allele and genotype frequencies for all *ARTS1* SNP were in Hardy-Weinberg equilibrium. We observed a significant increase in the minor allele frequency of rs27044 in the AS group compared to controls. The minor allele frequencies of rs10050860 and rs2287987 showed a significant decrease in AS patients compared to controls. No significant difference was found in allele frequencies of rs30187 and rs17482078 between the AS and the control group.

Genotype distributions showed significant differences between AS and control groups for rs27044, rs10050860, and rs2287987. For the rs10050860 and rs2287987 variants, the prevalence of homozygosity for minor alleles (TT, CC, respectively) showed a more than 3-fold increase in the control group compared to AS patients. For rs17482078 and rs30187 no significant difference was observed in the distribution of genotypes between the AS patients and controls after statistical correction (Table 1).

Pairwise linkage disequilibrium was calculated to reflect cosegregation between 2 SNP. We found no cosegregation between any of the 5 *ARTS1* SNP investigated in our population sample (data not shown).

The GCCTT and GCCCT haplotypes were associated with a risk for AS in Hungarian patients ($p = 0.0001$, OR 2.36, and $p = 0.033$, OR 1.83, respectively), while the CCCTC haplotype was associated with protection against AS ($p = 0.013$, OR 0.47; Table 2).

Patient gender, presence of peripheral arthritis or extraskeletal manifestations, and age at first complaint were unrelated to any of these *ARTS1* SNP in the AS group (data not shown).

From the 297 AS patients, 231 were found to be HLA-B27-positive. We observed 2 HLA-B27 subtypes in the AS patients: B*2705 and B*2702. However, only the B*2705 subtype was significantly associated with AS when we compared the data with HLA-B27 subtypes of healthy individuals (Table 3).

We examined the possible connection between HLA-B27 subtype and allele frequencies of the 5 *ARTS1* SNP. In our population sample, carriage of the G allele of the rs27044 was significantly associated with the B*2705 subtype in patients with AS ($p = 0.009$, OR 2.72, 95% CI 1.27–5.84). Other alleles of the 5 investigated *ARTS1* SNP showed no significant association with the HLA-B27 subtype.

Table 1. Allele and genotype frequencies of *ARTS1* polymorphism in patients with AS and controls. A Bonferroni corrected $p < 0.01$ was considered significant.

SNP	AS, n = 297 n (%)	Control, n = 200 n (%)	p
rs27044			
Allele			0.001
G	190 (31.9)	88 (22.0)	
C	404 (68.0)	312 (78.0)	
Genotype			0.0004
GG	27 (9.1)	14 (7.0)	
GC	136 (45.8)	60 (30.0)	
CC	134 (45.1)	126 (63.0)	
rs17482078			
Allele			0.044
T	95 (15.9)	84 (21.0)	
C	499 (84.0)	316 (79.0)	
Genotype			0.017
TT	5 (1.7)	13 (6.5)	
TC	85 (28.6)	58 (29.0)	
CC	207 (69.7)	129 (64.5)	
rs10050860			
Allele			0.006
T	95 (15.9)	92 (23.0)	
C	499 (84.0)	308 (77.0)	
Genotype			0.002
TT	4 (1.3)	14 (7.0)	
TC	87 (29.3)	64 (32.0)	
CC	206 (69.4)	122 (61.0)	
rs30187			
Allele			0.051
T	226 (38.0)	128 (32.0)	
C	368 (61.9)	272 (68.0)	
Genotype			0.089
TT	45 (15.1)	26 (13.0)	
TC	136 (45.8)	76 (38.0)	
CC	116 (39.1)	98 (49.0)	
rs2287987			
Allele			0.002
C	101 (17.0)	100 (25.0)	
T	493 (82.9)	300 (75.0)	
Genotype			0.0013
CC	4 (1.3)	14 (7.0)	
CT	93 (31.3)	72 (36.0)	
TT	200 (67.3)	114 (57.0)	

DISCUSSION

Ankylosing spondylitis is a chronic inflammatory disease of the axial skeleton manifested by back pain and progressive stiffness of the spine. Understanding of the pathogenesis of AS is limited; genetic and environmental factors are known to play an important role. Genome-wide screening approaches have been applied to determine chromosomal sites that are associated with disease in families with more than one affected member. A strong linkage to the MHC locus where HLA-B alleles are located has been confirmed. The strongest linkage with non-HLA susceptibility loci is at chromosome 16q, with lower linkages at multiple other chromosomes[5,32]. The WTCCC study, using Caucasian subjects of European ancestry, identified 2 non-HLA-B27 AS genes, *IL23R* and *ARTS1*, responsible for 26% and 9%, respectively, of the population-attributable risk[2]. The associ-

Table 2. ARTS1 haplotypes associated with AS.

	GCCTT OR (95% CI)	p	GCCCT OR (95% CI)	p	CCCTC OR (95% CI)	p
AS patients	2.36 (1.52–3.65)	0.0001*	1.85 (1.05–3.26)	0.033*	0.47 (0.26–0.85)	0.013*
B27+ AS patients	2.63 (1.65–4.17)	0.0001*	1.36 (0.79–2.35)	0.264	0.33 (0.14–0.79)	0.012*

* $p < 0.05$.

Table 3. HLA-B27 subtypes in Hungarian AS patients and HLA-B27-positive controls. $p < 0.05$ considered significant.

Subtype	HLA-B27-positive Controls, n = 70 n (%)	HLA-B27-positive AS Patients, n = 231 n (%)	p
B*2702	14 (20.0)	65 (28.14)	0.175
B*2705	32 (45.71)	166 (71.86)	< 0.001
B*2704	2 (2.86)	0	
B*2707	3 (4.29)	0	
B*2709	1 (1.43)	0	
B*2717	1 (1.43)	0	
B*2718	2 (2.86)	0	
B*2719	1 (1.43)	0	
B*2723	2 (2.86)	0	
B*2727	2 (2.86)	0	
B*2729	4 (5.71)	0	
B*2733	1 (1.43)	0	
B*2735	1 (1.43)	0	
B*2740	1 (1.43)	0	
B*2747	3 (4.29)	0	

ation with *IL23R* was confirmed by studies in Spain, Canada, and Hungary[33-35]; and that with *ARTS1* in Caucasian[12,13] and Korean[14] populations.

ARTS1 encodes an endoplasmic reticulum aminopeptidase that is involved in trimming peptides to optimal length for class I MHC presentation. *ARTS1* variants that lead to changes in aminopeptidase function could thus cause abnormalities in peptide presentation that might explain the association of *ARTS1* with the disease[36]. We examined the possible effect of 5 *ARTS1* SNP on the prevalence of AS in a Hungarian cohort. We studied the rs27044, rs17482078, rs10050860, rs30187, and rs2287987 SNP recently identified by the WTCCC study as risk-conferring variants for AS[2]. In our Hungarian population sample, rs27044, rs10050860, and rs2287987 variants were significantly associated with AS. Lack of significant difference of rs30187 and rs17482078 variants between AS and control subjects might be explained by the relatively small sample size and power values for these SNP (0.61 and 0.64, respectively). Haplotypic analysis revealed the association of 2 *ARTS1* haplotypes that increase risk of AS in the Hungarian population. These haplotypes are only partially in accord with previously reported *ARTS1* haplotypes in Caucasian and Korean samples[12-14] that can be explained by population differences.

IL23R, the other new possible AS candidate gene, encodes a cytokine receptor in the Th17 subset of T cells. Genetic variation in *IL23R* has been demonstrated to affect susceptibility to Crohn's disease[15,16], psoriasis[17], and AS[2]. The biological influence of the genetic variants in *IL23R* on expression and functionality is unknown, but it appears that the SNP represent an important link in the development of AS. Several mechanisms can be suggested by which polymorphisms can change the function of the receptor, as follows. The SNP located in the 3'-UTR can possibly cause overexpression of the receptor by increasing mRNA stability. The intronic polymorphisms could perhaps exert their influence by regulating the differential splicing[37]. Recently, Sáfrány, *et al* confirmed the effect of *IL23R* polymorphisms on the development of AS in a Hungarian population study[35]. Our findings, together with data from Sáfrány, *et al*[35], suggest that the biological significance of these non-MHC polymorphisms in the pathogenesis of AS is of great importance.

Other candidate genes have also been proposed. These include the cytochrome *CYP2D6* genotype and the IL-1 gene cluster[38,39]. The CYP2D6 poor-metabolizer phenotype can be due to at least 15 different genetic variants of the *CYP2D6* gene. A significant association between poor-metabolizer phenotype and AS has been reported[21,22]. A metaanalysis of studies of IL-1 gene cluster polymorphisms that included a total of 2675 patients with AS and 2592 healthy controls from 10 countries showed that the *IL1A* gene has the strongest linkage of those in the IL-1 gene cluster, but variation of the *IL1A* gene carries a risk of only 4% to 6%[20]. Several other genes have also been investigated as potential candidate genes for AS, without confirmation to date: *ANKH*[40,41], *TLR4*[42-45], *CARD15*[46,47], *TNF-alpha*[48], and *KIR*[49].

The only locus definitively linked to the spondyloarthropathies is HLA-B, and more specifically the HLA-B27 allele. HLA-B27 is present in about 95% of AS patients in the United States, Europe, and China, where the population prevalences of HLA-B27 are 3% to 8%, 9%, and 8%, respectively[50]. By comparison, both HLA-B27 and AS are virtually absent in certain native populations. In AS, HLA-B27 is estimated to contribute between 16% and 50% of the total genetic risk. At least 31 alleles of HLA-B27 have been characterized. Some of the alleles are silent mutations generating the same proteins[25].

B*2705 is the ancestral subtype and is by far the most frequent subtype found in Caucasians; it is highly associated with AS. B*2705 is further subdivided into B*27052,

B*27053, B*27054, B*27056, and B*27056 by silent substitutions. B*2704 is also a major subtype in frequency. It is the predominant subtype in Chinese and Japanese and is also associated with AS. B*2702, a major subtype in Mediterranean populations, is associated with AS as well. B*2701 to B*27010, B*2714, and B*2719 are much less frequent than B*2704 or B*2705; AS has been reported in each of these subtypes. The subtypes reported to be most weakly associated with AS are B*2706 and B*2709[51-54].

We assessed HLA-B27 subtype frequencies in Hungarian patients with AS, and compared frequencies with those of healthy controls. We observed 2 HLA-B27 subtypes, B*2705 and B*2702, in patients with AS. These subtypes were the most frequent among healthy subjects as well, and only the B*2705 was associated significantly with AS in Hungarians.

The difference in disease associations of HLA-B27 subtypes may result from an ability to bind the same peptide in different conformations. Protein misfolding of HLA-B27 is another possible explanation for the observation that B27 heavy chains in the absence of $ß_2$-microglobulin could promote arthritis[55,56]. This mechanism involves a proinflammatory response to overloading of the endoplasmic reticulum (ER) with misfolded proteins. When unfolded proteins overaccumulate in the ER, they induce a signal known as the "ER unfolded protein response" that leads to activation of nuclear factor-κB and subsequent generation of proinflammatory arthritis-causing cytokines. In patients with spondyloarthropathy, there is some evidence of an unfolded protein response in synovial fluid mononuclear cells[57]. A possible infectious contribution to AS has been pursued, but the data to date do not establish any particular infection as a trigger or cause. While HLA-B27 does not appear to have a unique role in allowing bacterial antigens or DNA access to joints, the presence of HLA-B27 may affect the host response to bacterial components.

The role of *ARTS1* in the pathogenesis of AS remains to be clarified. *ARTS1* is involved in trimming peptides to optimal length in the ER for MHC class I presentation. Abnormalities in peptide presentation together with certain forms of the HLA-B27 antigen may lead to the development of AS. We observed a significant association of the rs27044 variant of the *ARTS1* gene and the HLA-B*2705 subtype in Hungarians. This association might be relevant in understanding the pathogenesis of AS.

In agreement with recent studies, we confirmed the associations of certain *ARTS1* polymorphisms with AS in a Hungarian population study, and demonstrate for the first time that *ARTS1* variants and HLA-B27 contribute collectively to disease development in AS. Considering the indications for the association between *ARTS1* and AS in our Hungarian cohort as well as UK and US cohorts, these results suggest that this association is population-independent and contributes strongly to susceptibility to AS.

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