Protective effect of an ERAP1 haplotype in ankylosing spondylitis: investigating non-MHC genes in HLA-B27-positive individuals

Bruno Filipe Bettencourt1,2, Fabiana Leal Rocha1,2, Helena Alves3, Rosa Amorim4, Joana Caetano-Lopes5, Elsa Vieira-Sousa5,6, Fernando Pimentel-Santos7,8, Manuela Lima2,9, Grac¸ a Porto10,11, Jaime C. Branco7,8, João Eurico Fonseca5,6 and Jácome Bruges-Armas1,2

Abstract

Objective. The association of non-MHC genes with AS has been recently suggested. We aimed to investigate the association of the ERAP1, IL23R and TNFSF15 regions and the susceptibility to and protection from AS in HLA-B27-positive individuals.

Methods. A total of 200 unrelated AS patients and 559 healthy unrelated subjects, all HLA-B27 positive, were tested. Twenty single nucleotide polymorphisms (SNPs) were investigated in and near IL23R (nine SNPs), in ERAP1 (five SNPs) and in TNFSF15 (six SNPs).

Results. ERAP1 rs30187 (odds ratio (OR) = 1.5, P = 4.7 × 10⁻³) had the strongest association with AS susceptibility. A protective effect was found in three of the ERAP1 SNPs: rs17482078 (OR = 0.7, P = 2.8 × 10⁻²), rs10050860 (OR = 0.7, P = 2.3 × 10⁻²), rs2287987 (OR = 0.6, P = 1.3 × 10⁻²). The ERAP1 haplotype rs17482078/rs10050860/rs30187/rs2287987-CCTT showed an association with AS susceptibility (P = 6.8 × 10⁻³) and a protective effect was identified in rs17482078/rs10050860/rs30187/rs2287987-TTCC (P = 3.1 × 10⁻³). Significant association with AS susceptibility was found in one IL23R marker (rs1004819, P = 4.3 × 10⁻², OR = 1.3). No associations were observed in the TNFSF15 region.

Conclusion. The identification of a new protection haplotype in ERAP1 and the lack of association of the TNFSF15 region can provide new insights into the understanding of the mechanisms underlying the susceptibility to and protection from AS.

Key words: ankylosing spondylitis, ERAP1, IL23R, TNFSF15, protection haplotype.

Introduction

AS is an inflammatory rheumatic disease that is included in the spondyloarthritis group of diseases (SpA) and affects predominantly the axial skeleton—sacroiliac joints and spine. Disease heredity exceeds 90% and the MHC region, particularly HLA-B27, is the main contributor for this proportion, with an attributable risk near 50% [1–3].

New evidence has been obtained from genome-wide and whole-genome association studies (WGS). Those studies allowed a wider view of genetic factors related to AS, supporting the presence of non-MHC genetic susceptibility factors and consequently spreading the research to other areas outside MHC.
with AS was identified on chromosomes 1p, 2q, 6p, 9q, 10q, 16q and 19q [4]. Later, three other major studies narrowed down the chromosome areas first reported and allowed the identification of specific gene regions that could lead the quest for knowledge about the mechanisms underlying disease susceptibility [3, 5, 6].

The endoplasmic reticulum aminopeptidase 1 (ERAP1) and IL-23 receptor (IL23R) genes were two of the first mentioned genes outside the MHC region [3, 5]. ERAP1 molecules are encoded in the endoplasmic reticulum lumen [7] and are MHC class I related, playing an important role in peptide trimming for presentation at the cell surface [8]. The relation between ERAP1 and class I MHC loci may be the key for comprehension of the role played by HLA-B27 in susceptibility to AS. ERAP1 is also involved in the cleavage of cytokine cell surface receptors [9].

IL23R is one of the two subunits of the IL-23 receptor complex, present on IL-23-responsive cells and binds to IL-23, mediating its activity [10]. The IL23R gene encodes a critical cytokine receptor in the Th17 lymphocyte subset. Th17 cells have been identified as mediators of the inflammatory process in several models of autoimmunity [11, 12].

The association between a haplotype composed by single nucleotide polymorphisms (SNPs) near the TNF ligand superfamily member 15 gene (TNFSF15) and SpA was recently reported [13]. Data showed that D9S1824, at 115.9 Mb from the p-telomere, and D9S1682 were markers of statistically significant linkage. The D9S1682 region had been reported earlier in a WGS as a suggestive linkage peak for AS [4]. The ligand-receptor pairing of TNFSF15-DR3 was pointed out as a regulator of Th17 differentiation and activation. This fact may link the TNFSF15 region and AS, since an increased level of Th17 cells in AS patients, when compared with healthy controls, had already been reported [14]. Therefore, targeting the TNFSF15-DR3 pathway could provide new insights into the role of TNFSF15 in AS onset and progression.

Together, HLA-B27, ERAP1 and IL23R may be responsible for a total attributable risk of 84% [3]. Consequently individuals from AS families or from the general population carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives carrying some of the minor alleles of these non-MHC genes should have a higher risk of developing the disease. The prevalence of AS is higher in HLA-B27 relatives of AS patients compared with the general population [15], although there is no clear explanation for those relatives executing the modified New York criteria [16], were included in the study. The DNA samples were collected from outpatients in several hospitals and medical centres from central and southern Portugal as well as the Azores. The control group was composed by 559 healthy unrelated subjects, all HLA-B27 positive and >35 years old. Most of the controls (n = 519) were healthy, HLA-B27-positive, Portuguese bone marrow donors from the Portugal mainland. The remaining controls were recruited among Azorean HLA-B27-positive unrelated individuals from the Autoimmune Disease Department of Hospital de Santo Espírito da Ilha Terceira (n = 40). All subjects were of Portuguese descent. There was an overlap of five AS patients between this study and a previous Portuguese report [17]. Written informed consent was obtained from each participant. The study was approved by the ethics committee of Hospital de Santo Espírito da Ilha Terceira.

Genotyping
Genomic DNA was extracted from peripheral blood cells according to standard procedures. A total of 20 SNPs were investigated in and near IL23R (nine SNPs), in ERAP1 (five SNPs) and near TNFSF15 (six SNPs) (Table 1). All samples were genotyped using Taqman SNP genotyping assays (Applied Biosystems, Foster City, CA, USA) performed as described by the manufacturer. Genotyping reactions were carried out with an ABI 7500 fast thermocycler. The allele call was obtained through AB software v2.0.5 analysis of allelic discrimination plots.

Statistical analysis
The Hardy–Weinberg equilibrium (HWE) was calculated by the default method implemented in PLINK 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/) [18, 19]. Genotyped SNPs were assessed for missingness. Individuals or markers with >10% missingness were excluded from the remaining calculations.

SNP association analyses were performed using the Cochrane–Armitage test of trend as implemented in PLINK 1.07. Imputation analyses were carried out using Markov chain haplotyping software—MaCH 1.0 (http://www.sph.umich.edu/csg/abecasis/MACH/) [20, 21]. The SNP marker map and haplotype phased data used as a reference for SNP imputation was from the CEU individuals (Utah residents with northern and western European ancestry from the CEPH collection) from the International HapMap Project (phase III, release 3, May 2010) on NCBI B36 dbSNP b126 IL23R. Only SNPs that were either genotyped or had a relatively high imputation quality score (R2 > 0.3) were analysed. Recombination rates and SNP association plotting were obtained with LocusZoom 1.1 [22].

Odds ratios (ORs) with a 95% CI were calculated for the SNPs’ minor alleles. OR > 1 indicates a susceptibility allele and OR < 1 indicates a protective allele. Pairwise D’ and linkage disequilibrium (LD) correlation coefficient (r2) values, as well as haplotype analysis, were calculated by Haplovlew 4.2 [23]. The LD blocks were determined by
TABLE 1 SNPs genotyped in IL23R, ERAP1 and region near TNFSF15

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Gene</th>
<th>SNP</th>
<th>Position, bp</th>
<th>Gene location</th>
<th>Alleles (Taqman assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IL23R</td>
<td>rs1004819</td>
<td>67442801</td>
<td>Intron</td>
<td>A/G</td>
</tr>
<tr>
<td>1</td>
<td>IL23R</td>
<td>rs10489629</td>
<td>67460937</td>
<td>Intron</td>
<td>C/T</td>
</tr>
<tr>
<td>1</td>
<td>IL23R</td>
<td>rs2201841</td>
<td>67466790</td>
<td>Intron</td>
<td>A/G</td>
</tr>
<tr>
<td>1</td>
<td>IL23R</td>
<td>rs11465804</td>
<td>67475114</td>
<td>Intron</td>
<td>G/T</td>
</tr>
<tr>
<td>1</td>
<td>IL23R</td>
<td>rs11209026</td>
<td>67478546</td>
<td>Exon</td>
<td>A/G</td>
</tr>
<tr>
<td>1</td>
<td>IL23R</td>
<td>rs1343151</td>
<td>67491717</td>
<td>Intron</td>
<td>A/G</td>
</tr>
<tr>
<td>1</td>
<td>IL23R</td>
<td>rs10889677</td>
<td>67497708</td>
<td>Exon</td>
<td>A/C</td>
</tr>
<tr>
<td>1</td>
<td>IL23R</td>
<td>rs11209032</td>
<td>67512680</td>
<td>Intergenic</td>
<td>A/G</td>
</tr>
<tr>
<td>1</td>
<td>IL23R</td>
<td>rs1495965</td>
<td>67526096</td>
<td>Intergenic</td>
<td>C/T</td>
</tr>
<tr>
<td>5</td>
<td>ERAP1</td>
<td>rs27044</td>
<td>96144608</td>
<td>Exon</td>
<td>C/G</td>
</tr>
<tr>
<td>5</td>
<td>ERAP1</td>
<td>rs17482078</td>
<td>96144622</td>
<td>Exon</td>
<td>C/T</td>
</tr>
<tr>
<td>5</td>
<td>ERAP1</td>
<td>rs10050860</td>
<td>96147966</td>
<td>Exon</td>
<td>C/T</td>
</tr>
<tr>
<td>5</td>
<td>ERAP1</td>
<td>rs301817</td>
<td>96150086</td>
<td>Exon</td>
<td>C/T</td>
</tr>
<tr>
<td>5</td>
<td>ERAP1</td>
<td>rs2287987</td>
<td>96155291</td>
<td>Exon</td>
<td>C/T</td>
</tr>
<tr>
<td>9</td>
<td>TNFSF15</td>
<td>rs7849556</td>
<td>116522493</td>
<td>Intergenic</td>
<td>A/C</td>
</tr>
<tr>
<td>9</td>
<td>TNFSF15</td>
<td>rs10817669</td>
<td>116522836</td>
<td>Intergenic</td>
<td>A/G</td>
</tr>
<tr>
<td>9</td>
<td>TNFSF15</td>
<td>rs10759734</td>
<td>116536471</td>
<td>Intergenic</td>
<td>A/G</td>
</tr>
<tr>
<td>9</td>
<td>TNFSF15</td>
<td>rs6478105</td>
<td>116557006</td>
<td>Intergenic</td>
<td>A/G</td>
</tr>
<tr>
<td>9</td>
<td>TNFSF15</td>
<td>rs10982396</td>
<td>116558750</td>
<td>Intergenic</td>
<td>C/G</td>
</tr>
<tr>
<td>9</td>
<td>TNFSF15</td>
<td>rs10733612</td>
<td>116562871</td>
<td>Intergenic</td>
<td>C/T</td>
</tr>
</tbody>
</table>

Results

The study had 80% power to detect an association for an OR between 1.5 and 1.7. The patient group was 62% males and 38% females, with a mean age of 48.2 ± 14.5 years (Table 2). The subjects included in the control group were all ≥35 years old and were 42% males and 58% females. A total of 27 controls and 8 patients were excluded from the remaining calculations, since they presented an overall missing rate >10%. The same rate was observed for the marker rs10982396 (TNFSF15), which was consequently removed from the association calculations.

SNPs were in HWE (P > 0.05) in the control group. The MAF values were all >0.01 in both groups (Table 3). A significant association was found with one of the IL23R markers—rs1004819 (OR = 1.3, P = 4.3 × 10⁻³) with an attributable risk of 10.1%. None of the remaining eight SNPs genotyped in the IL23R region showed a significant association with AS (Table 3). The same lack of association (P > 0.05) was observed in all 45 SNPs that were considered after imputation (R² ≥ 0.3) in and around IL23R (Fig. 1A).

Single-marker association was observed in all genotyped SNPs in the ERAP1 gene. The marker rs30187 revealed the strongest association level (OR = 1.5, P = 4.7 × 10⁻³) (Table 3). The attributable risk for rs30187 and rs22047 (OR = 1.6, P = 3 × 10⁻²) was 11.7% and 14.6%, respectively. A protective effect was found in three of the ERAP1 SNPs—rs17482078 (OR = 0.7, P = 2.8 × 10⁻²), rs10050860 (OR = 0.7, P = 2.3 × 10⁻²) and rs2287987 (OR = 0.6, P = 1.3 × 10⁻²). Fifty-five SNPs were imputed in the ERAP1 region, in a block of ~89 kb (Fig. 1B). Thirty-two of those SNPs had a significant association with AS. The strongest nominal association was with rs41135 (96191782 bp, R² = 0.46, P = 2.3 × 10⁻⁴), with a level of significance higher than any of the genotyped SNPs included in the study. No association was observed in the TNFSF15 region in either the genotyped or in the nine imputed SNPs (Fig. 1C).

The results of the analysis of haplotypes and pairwise D’ values are presented in Fig. 2. An LD block containing rs17482078/rs10050860/rs30187/rs2287987 (Fig. 2B)
was identified in ERAP1. Two of the haplotypes in ERAP1 revealed a significant association with AS. The haplotype −CCTT was associated with susceptibility to AS \( (P = 6.8 \times 10^{-5}) \), while its complementary haplotype −TTCC showed a protective effect \( (P = 3.1 \times 10^{-2}) \). The logistical regression analysis revealed that there was still a marginal omnibus association \( (P < 0.05) \) after controlling for each one of the ERAP1 SNPs included in the LD blocks. There was no significant \( P \)-value \( (P = 0.14) \) when the ERAP1 haplotype −CCTT was controlled. On the other hand, the omnibus test remained significant after controlling for −TTCC \( (P = 2.3 \times 10^{-5}, OR = 0.68) \). An LD block containing rs7849556/rs10817669/rs10759734 was identified in the TNFSF15 region (Fig. 2C), but no association with AS was found with the TNFSF15 haplotypes (Table 4). The \( R^2 \) values of the haplotypic blocks were between 96 and 100, showing strong or complete LD.

**Discussion**

We have confirmed the genetic association between AS and both ERAP1 and IL23R as previously reported [6, 17, 26–30]. All the ERAP1 SNPs presented a significant association with AS. The rs30187 minor allele registered the highest level of susceptibility to AS \( (P = 4.7 \times 10^{-5}) \), in accordance with the already known data from the Portuguese population [17]. The attributable risk for this marker in both studies is also similar, 11.7% and 9.1%, respectively. rs30187 (Arg528Lys) was the only coding marker within a SNP block identified in a group composed of Australian, British and North American individuals of European descent [5] and showed a strong association in another large Caucasian cohort \( (P = 1.8 \times 10^{-2}) \) [6]. Modelling of the ERAP1 protein pointed to the presence of Arg528 at the mouth of the putative enzyme substrate pocket. These results, as well as data from family-based studies [31], support rs30187 as one of the ERAP1 variants with the strongest association to AS.

The significant association found in rs27044 \( (P = 3 \times 10^{-5}) \) was also in accordance with the previous Portuguese report and other Caucasian replicates [3, 17]. Despite the lower association level observed in rs27044, when compared with rs30187, this marker has a higher attributable risk in our group (14.6%). These two SNPs were the only markers associated with AS in both Caucasian and Han Chinese patients [26, 28, 32].

ERAP1 rs17482078, rs10050860 and rs2287987 were in very strong LD \( (R^2 > 0.97) \). These markers revealed a protective effect in our HLA-B27 population (Table 3), with a preventive fraction between 6% and 7%. The MAF values for these three SNPs are in accordance with that already reported in a Portuguese and a Spanish group [17, 33]. Despite the similar MAF values, no significant association was observed in the Portuguese group. This lack of association was also reported in Han Chinese and Hungarian groups [26, 34].

Two of the three haplotypes found in ERAP1 had a significant association with AS (Table 4). The haplotype −CCTT \( (P = 6.8 \times 10^{-5}) \) was associated with AS susceptibility. This haplotype, is part of a haplotype identified in Han Chinese \( (P = 4.71 \times 10^{-5}) \) as well as of another haplotype identified in a Canadian case–control study \( (P = 7 \times 10^{-8}) \), both with a strong association to AS susceptibility [26, 28]. However, the logistic regression results revealed that this association is not significant when controlling for this haplotype. The other haplotype, −TTCC \( (P = 3.1 \times 10^{-2}) \) had a protective effect. This haplotype is exactly complementary to the susceptibility haplotype −CCTT. To our knowledge there is no
Fig. 1 Genotyped and imputed SNP association plots.

Distances are measured from the p-telomere, according to the National Center for Biotechnology Information Reference. Association results are plotted as $-\log_{10}(P\text{-value})$ on the left-hand y-axis. $-\log_{10}(P = 0.05) = 1.3$. Filled circles: imputed SNPs. Filled diamonds: genotyped SNPs. LD is reported in the colour scale. Blue line and right hand y-axis represent the recombination rate (cM/Mb as per HapMap data). The positions of the exons are indicated below the x-axis. The arrow indicates the direction of translation. (A) SNP plot for IL23R; LD is relative to rs1004819. (B) SNP plot for ERAP1; LD is relative to rs30187. (C) SNP plot for the region near TNFSF15; LD is relative to rs10759734.
previous report mentioning this haplotype and its protective effect in HLA-B27-positive healthy individuals. The logistic regression results confirmed these results, since they remained significant after controlling for this haplotype. We also concluded that none of the SNPs have an independent effect, due to the results obtained in the logistical regression test.

In terms of protein structure, this protection haplotype has the following correspondence: ERAP1-Gln725/Asn575/Arg528/Val349. The crystal structure of ERAP1 has recently been published, revealing the location of these altered residues around the substrate binding and regulatory sites [35, 36]. It was shown that rs30187 wild-type (Lys528) ERAP1 protein was able to degrade a 13-mer peptide in such an efficient way that the starting peptide, with a four amino acid N-terminal extension, was barely detected within 30 min [36]. However, when the Arg528 variant was tested, a less efficient peptide processing was observed, suggesting some failure in the trimming process stopping at the 11-mer [36].

In accordance with a previous report, where the interaction between ERAP1 and HLA-B27 was tested [6], the SNPs rs17482078 (Arg725Gln) and rs10050860 (Asp575Asn) were found to be in complete LD. Another in vitro examination showed that, like with Arg528 (rs30187), the Gln725 (rs17482078) alleles had ~40% slower rates of substrate trimming than wild-type ERAP1, suggesting once more a loss of peptide processing function. However, the proteolytic rate of Asn575 (rs10050860) was similar to wild-type ERAP1 [6]. Val349 is located in the active site of the ERAP1 molecule [35]. This variant can modulate hydrolysis, substrate binding or the conformational transition between the open and closed states of the ERAP1 molecule, which is critical for enzymatic activity [37].

Wild-type ERAP1 molecules are able to produce peptides containing eight to nine residues from longer precursors that have been partially processed by the proteosome [38]. These peptides have the optimal length for HLA class I binding and presentation [38]. However, as
mentioned above, the presence of the molecular variants that compose the ERAP1 protection haplotype, found during this work, decrease this trimming activity, excluding the rs30187 allele. The resulting peptides from this deficient activity are longer than the typical molecules to which HLA class I bind. The protection ERAP1 haplotype found in this study was identified in an HLA-B27-positive population. In addition, a gene–gene interaction study between HLA-B27 and ERAP1 revealed that the ERAP1 association with AS was only observed in HLA-B27-positive patients [6].

These findings are fully consistent with a recent report where the interaction between ERAP1 variants and HLA-B27 was investigated in human cells [37]. The results of this in vivo study revealed that less active ERAP1 variants influence the number and length of HLA-B27 ligand peptides. Moreover, it was shown that these variants have a global influence on the B*27:04 peptidome, affecting the relative expression of many B*27:04 ligands as a function of peptide size [37].

The already known data support the hypothesis that reduced trimming of peptides and consequent altered antigen presentation on HLA-B27 molecules is an important mechanism involved in AS development and protection.

The association of IL23R rs10889677, formerly described in the Portuguese population, was not identified in this study (P = 0.40) [17]. Moreover, our results show that the MAF in patients is similar to that in controls (0.35 vs 0.31). The only association observed in IL23R was with rs1004819 (P = 4.3 × 10^{-7}). This association had been found previously in the Portuguese population, with a similar MAF but in a lower level of magnitude [17]. The results obtained for rs1004819 are also in accordance with a Hungarian population study performed with a small number of samples [30]. The authors were able to register some significant associations and demonstrate that rs1004819 was the IL23R SNP with the strongest association with AS in that population. They also indicated that the presence of rs1004819 allele A increases the risk for AS by more than 2-fold [30]. Additionally, the attributable risk for rs1004819 (10.1%) is similar to that reported in the other Portuguese cohort (11%) [17]. One of the first WGSs in AS, reported an analogous attributable risk for rs11209032 (9%) in Caucasians (British and North American) [3]. This marker revealed the strongest association with AS in that study. The differences between our IL23R results and those of the previous study concerning a Portuguese population can be partially explained by the modest power of both studies concerning this gene. The use of controls that are HLA-B27 positive has no influence when testing IL23R markers, since it is stated that HLA-B27 status has no real relationship with the function of IL23R [30, 39]. Despite all the available knowledge about IL23R, the biological impact of rs1004819 variants on the expression and function of this gene is currently unknown.

An association between several SNPs near the TNFSF15 gene and SpA was reported in a comprehensive linkage and association analyses study [13]. The data from this study showed two areas of statistically significant linkage, supporting the validity of linkage between this region and SpA. Haplotype research also confirmed this tendency and revealed a significant strong association of the individual haplotype rs7849556/rs10817669/rs10759734/rs6478105/rs10982396/rs10733612–CGGGGT with SpA [13]. In our study, none of the markers near TNFSF15 revealed a significant association with AS (P > 0.3). We have identified three haplotypes in a block with high D’, however, none of them presented an association with AS (Table 4). The differences between both studies can be explained by a different analysis methodology, as the previous study explored SpA as a whole and we tested AS alone. To our knowledge, this was the first time that markers near the TNFSF15 gene were tested only in AS and with an HLA-B27 population. Despite the previously reported association of the TNFSF15 region with SpA, this effect was not confirmed when testing AS alone.

In summary, we emphasize that the protective ERAP1 haplotype found in our study was identified in a healthy HLA-B27 population. The identification of the protection haplotype rs17482078/rs100508607/rs30187/rs2287987–TTCC is a finding that can provide new insights in the understanding of the relation between ERAP1 and HLA-B27 molecules and its contribution to the susceptibility to and protection from AS.

Rheumatology key messages

- The ERAP1 –TTCC haplotype has a protective effect for AS in HLA-B27-positive individuals
- ERAP1 and IL-23R are associated with AS susceptibility in the Portuguese population.
- The TNFSF15 region is not associated with AS susceptibility.

Funding: This work was partially funded by a grant from the DRCT (Science and Technology Regional Board) from the Autonomous Government of Azores, Portugal (DRCT M2.1.2/I/014/2007).

Disclosure statement: The authors have declared no conflicts of interest.

References

18. Purcell S, Neale B, Todd-Brown K et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75.
