Association of *IL23R* and *ERAP1* genes with ankylosing spondylitis in a Portuguese population

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Abstract

Objective

Association between ankylosing spondylitis (AS) and two genes, ERAP1 *and* IL23R*, has recently been reported in North American and British populations. The population attributable risk fraction for* ERAP1 *in this study was 25%, and for* IL23R*, 9%. Confirmation of these findings to* ERAP1 *in other ethnic groups has not yet been demonstrated. We sought to test the association between single nucleotide polymorphisms (SNPs) in these genes and susceptibility to AS among a Portuguese population. We also investigated the role of these genes in clinical manifestations of AS, including age of symptom onset, the Bath Ankylosing Spondylitis Disease Activity, Metrology and Functional Indices, and the modified Stoke Ankylosing Spondylitis Spinal Score.*

Methods

The study was conducted on 358 AS cases and 285 ethnically matched Portuguese healthy controls. AS was defined according to the modified New York Criteria. Genotyping of IL23R *and* ERAP1 *allelic variants was carried out with TaqMan allelic discrimination assays. Association analysis was performed using the Cochrane-Armitage and linear regression tests of genotypes as implemented in PLINK for dichotomous and quantitative variables respectively. A meta-analysis for Portuguese and previously published Spanish* IL23R *data was performed using the StatsDirect® Statistical tools, by fixed and random effects models.*

Results

A total of 14 nsSNPs markers (8 for IL23R*, 5 for* ERAP1*, 1 for LN-PEP) were analysed. Three markers (2 for* IL23R *and 1 for* ERAP1*) showed significant single-locus disease associations, confirming that the association of these genes with AS in the Portuguese population. The strongest associated SNP in* IL23R *was rs1004819 (OR=1.4, p=0.0049), and in* ERAP1 *was rs30187 (OR=1.26, p=0.035). The population attributable risk fractions in the Portuguese population for these SNPs are 11% and 9.7% respectively. No association was seen with any SNP in LN-PEP, which flanks* ERAP1 *and was associated with AS in the British population. No association was seen with clinical manifestations of AS.*

Conclusions

These results show that IL23R *and* ERAP1 *genes are also associated with susceptibility to AS in the Portuguese population, and that they contribute a significant proportion of the population risk for this disease.*

Key words

Ankylosing spondylitis, *ERAP1*, *IL23R*.

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disorder with an estimated prevalence of 0.1–0.9% in Caucasian populations (1, 2). Although the contribution of the HLA-B27 allele to the overall genetic predisposition has been estimated at 20-30% and the contribution of all genes in the HLA region estimated at 40-50% (3), genes outside the major histocompatibility complex are strongly implicated in the aetiology of the disease. In particular, the genes *IL23R* and *ERAP1* have recently been demonstrated to be associated with AS in British and North American Caucasians (4). The population attributable risk fraction for *ERAP1* in this study was 25%, and for *IL23R*, 9% (4). Association of the *IL23R* findings has recently been confirmed in Canadian (5) and Spanish populations (6), but not yet been demonstrated for *ERAP1*. We sought to test the association between single nucleotide polymorphisms (SNPs) in these genes and susceptibility to AS in the Portuguese population. We also investigated the role of these genes in the pattern of AS clinical manifestations, including age of symptom onset and the Bath Ankylosing Spondylitis Disease Activity (BASDAI) (7), Functional (BASFI) (8) and Metrology (BASMI) (9) Indices, and the modified Stoke Ankylosing Spondylitis Spinal Score (10).

Methods

Subject

The study group comprised 358 unrelated AS patients and 285 ethnically matched healthy controls. Individuals included in the study were of Portuguese ancestry and came from mainland Portugal. All cases were diagnosed as having AS according to the modified New York Criteria (11). Cases were recruited from hospital outpatient departments; controls were healthy Portuguese bone marrow donors. This study was approved by the Ethics Board of the involved centres, and written informed consent was obtained from the individuals involved in this study.

Patients completed a questionnaire containing a self-assessment of clinical features, including the BASDAI and the BASFI. Metrology was performed by one of the investigators (FS), to obtain the BASMI. Age at disease onset was defined as the age at onset of clinical symptoms. Similarly, disease duration was defined as the period (years) after the onset of clinical symptoms.

Genotyping

Genomic DNA from cases and controls individuals was prepared from peripheral blood lymphocytes using standard techniques. Samples were genotyped for the 14 nsSNPs markers used in the Wellcome Trust Case-Control Consortium/Australo-Anglo-American Spondyloarthritis Consortium (WTCCC/TASC) study (4). Eight SNPs were typed in and around *IL23R* (rs1495965, rs10489629, rs11465804, rs10889677, rs1343151, rs1004819, rs11209026, rs11209032), 5 in *ERAP1* (rs2287987, rs30187, rs10050860, rs27044, rs17482078) and 1 in *LN-PEP* (rs2303138). Taqman® SNP genotyping assays (Applied Biosystems, Foster City, USA) were used for genotyping, which was performed according to the manufacturers protocols (see Table I).

Genotyping reactions were performed with an AB 7900HT, and the allele call by the analysis of allelic discrimination plots with AB SDS 2.3 software. Replicate genotype known and negative control samples were typed in each 96 well plate.

Statistical analysis

SNP genotype data was assessed for missingness (overall, and differences , cases and controls assessed by χ^2 test) and for Hardy-Weinberg equilibrium in controls. Individuals with >10% missingness were excluded (n=5 controls, 2 cases).

Association analysis was performed using the Cochrane-Armitage test as implemented in PLINK (12). Association between SNPs and the quantitative variables age of symptom onset, BASDAI, BASFI, BASMI and mSASSS were tested by linear regression assuming an additive model using PLINK, taking into account gender and disease duration as covariates.

Imputation analyses were carried out using Markov Chain Haplotyping software

Table I. *IL23R*, *ERAP1* and *LN-PEP* genetic variants analysed in AS patients and controls. Positions are given as per dbSNP build 129.

Genes	Chromosome	Position (bp)*	Gene location	Taqman ASSAY ID	NCBI SNP Reference
IL23R	1	67442801	Intron	C_1272321_10	rs1004819
	1	67460937	Intron	C_30279129_20	rs10489629
	1	67475114	Intron	C_31222838_10	rs11465804
	1	67491717	Intron	C_8367043_10	rs1343151
	1	67497708	Exon	C_11283764_10	rs10889677
	1	67478546	Exon	C_1272298_10	rs11209026
	1	67526096	Intergenic	C_8361864_10	rs1495965
	1	67512680	Intergenic	C_2720238_10	rs11209032
ERAP1	5	96150086	Intergenic	C_3056885_10	rs30187
	5	96147966	Intergenic	C_3056876_10	rs10050860
	5	96155291	Intergenic	C_3056893_10	rs2287987
	5	96144608	Intergenic	C_3056870_10	rs27044
	5	96144622	Intergenic	C_3056871_10	rs17482078
LN-PEP	5	96376466	Missense Mutation	C_25649482_10	rs2303138

Table II. Characteristics of the Portuguese AS cases and controls*.

	AS patients (n= 358)	Controls (n=285)
Nº (%) males/ Nº (%) females	226 (63%) / 132 (37%)	127 (44.6%)/158 (55.4%)
Age, years	45.4 ± 13.3	35.9 ± 11.1
Disease duration, years	19.1 ± 12.6	
BASDAI	4.2 ± 2.3	
BASFI	4.1 ± 2.7	
BASMI	4.0 ± 2.5	
mSASSS	20.9 ± 22.9	

*Except where indicated otherwise, values are the mean ± SD (standard deviation). AS- Ankylosing spondylitis, BASDAI- Bath AS Disease activity Index; BASFI- Bath AS Functional Index; BASMI- Bath AS Metrological Index.

Table III. Frequency of *ERAP1* and *LNPEP* minor allele frequencies in the Portuguese AS cohort.

Gene	SNP	Minor Allele	Portuguese Cohort			
			Case MAF	Control MAF	OR	*p*-value
ERAP1	rs27044	G	0.37	0.32	1.26 (1.10-1.60)	0.044
ERAP1	rs17482078	T	0.16	0.19	0.78 (0.58-1.05)	0.096
ERAP1	rs10050860	T	0.16	0.20	0.76 (0.57-1.01)	0.057
ERAP1	rs30187	T	0.47	0.41	1.26 (1.01-1.57)	0.035
ERAP1	rs2287987	C	0.16	0.20	0.77 (0.58-1.03)	0.074
LNPEP	rs2303138	A	0.08	0.07	1.23 (0.80-1.88)	0.33

(MaCH; http://www.sph.umich.edu/csg/abecasis/MACH/) using phased data from CEU individuals from release 22 of the HapMap project as the reference set of haplotypes. We only analyzed SNPs that were either genotyped or could be imputed with relatively high confidence (R^2>=0.3).Association analysis of imputed SNPs was performed assuming an underlying additive model using the software package MACH2ASSOC (Li, Willer, Ding, Scheet and Abecasis, unpublished data) which accounts for uncertainty in prediction of the imputed data by weighting genotypes by their posterior probabilities.

A meta-analysis study was performed using StatsDirect® software (13), specifically to test the association of *IL23R* in the Iberian population, combining the Portuguese data presented here, and previously published Spanish data (6). Both fixed and random effects analysis was performed; non-combinability of studies was assessed using the Cochrane Q statistic, and the extent of heterogeneity between studies assessed using the I^2 statistic. Power calculations were performed using the Genetic Power Calculator (14).

Results

The Portuguese AS cohort population (Table II) included 228 (63.5%) men and 132 (36.5%) women with a mean age of 45.4 (±13.2 SD) years (range 20-79 years) and a mean disease duration of 19.1 (±12.6) years (range 0-60 years), of whom 82% were HLA-B27 positive. Epidemiological data of the cases and controls are summarized in Table II.

All the studied genetic markers were in Hardy-Weinberg equilibrium in the controls group, had missingess rates <10%, and none had differential missingness in cases and controls ($p<10^{-2}$). The minor allele frequencies (MAF) of the 14 SNPs are presented in Tables III and IV.

Single-marker association tests revealed significant AS associations for the *ERAP1* SNP rs30187 (odds ratio (OR) 1.26, p=0.035) and rs27044 (OR 1.26, p=0.044) (Table III). Unlike in the previous British and North American studies, no association was seen with the SNP rs2303138, which lies within *LN-PEP*, which flanks *ERAP1*. Of the 119 SNPs imputed in and around *ERAP1*, 32 demonstrated nominal association with AS (p<0.05), with the strongest association being with rs41135 (p=0.014) (Fig. 1).

Two SNPs in and around *IL23R* demonstrated significant association (rs1004819 OR=1.44, $p=4.9\times10^{-3}$; rs10889677 OR=1.41, $p=5.8\times10^{-3}$) (Table IV). The strongest associated SNP reported in both Crohn's disease (15) and psoriasis (16) (Arg381Gln; rs11209026) did not show any protective effect in our population. No imputed SNP was more strongly associated with AS than these two genotyped SNPs, but many SNPs in a block extending from rs10889667 as far as rs11465817 (67493685 bp from the p-telomere) were associated with AS with p<0.01 (Fig. 2). Of the 49 imputed SNPs in and around *IL23R*, 23 demonstrated nominal association with AS (p<0.05).

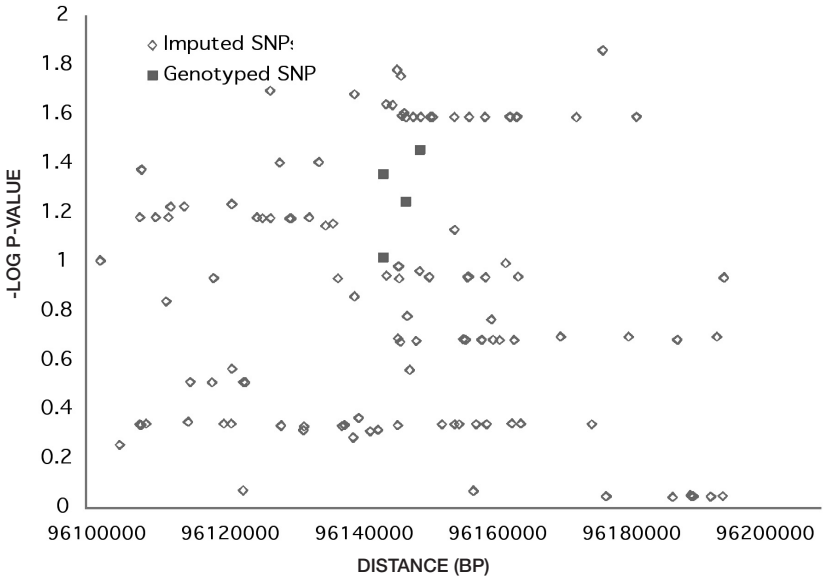

Fig. 1. Association results for imputed and directly genotyped SNPs in *ERAP1*. Distances are in base pairs (bp) from the p-telomere. Association significance is reported as -$\log_{10}$(*p*-values).

The population attributable risk fraction in the Portuguese population for rs1004819 is 11%, and for rs30187 is 9.7%.

The meta-analysis performed between Portuguese and Spanish populations (Table V) revealed significant AS associations, through the fixed effects, for SNPs rs1004819, rs11209026, and rs1343151. However, for markers rs11209026, rs1343151 and rs210889617 in particular, there were significant differences in the findings in the Spanish and Portuguese populations, reflected by significant Cochrane Q statistics (rs11209026, *p*=0.017; rs1343151, *p*=0.023; rs10889617, *p*=0.0005), suggesting that a random effects model should be applied. Considering the random effects models, the associations with AS remain significant for rs1004819.

No association was observed between *IL23R* or *ERAP1* variants and age of symptom onset, BASDAI, BASFI, BASMI or mSASSS (data not shown).

The study had 80% power to detect associations with these quantitative variables at a significance level of α=0.05 for SNPs contributing >4% of the trait variance, assuming linkage disequilibrium between the marker and disease-associated variant of D'>0.8 and that the marker and disease-associated allele frequencies are equal. Considering the case-control analysis of disease susceptibility, assuming a population prevalence of disease of 0.4%, minor allele frequencies of 0.1–0.5, and D'>0.8, the study had 80% power to detect an additive association with heterozygote odds ratio of 1.6-1.8.

Discussion

Many candidate genes outside the MHC have been evaluated in different studies regarding AS susceptibility and/or phenotype associations. Recently, association has been demonstrated and confirmed with SNPs in and around the genes *ERAP1* and *IL23R* in British and North American populations (4).

The *IL23R* association with AS was also recently replicated in the Canadian (5) and Spanish (6) populations. The present study has replicated this association in the Portuguese population. The peak association in our cohort is seen with rs1004819. This is different from the UK, United States, Canada and Spanish data sets, where the peak association was observed for different SNPs, although the minor allele frequencies (MAF) that we have observed for SNPs in Portuguese were similar to those reported in other British and North Americans. Furthermore, the association observed in the Portuguese population had a similar magnitude of effect to the one described in those other populations, as can be appreciated by the attributable risk for rs1004819, which is very similar to the one reported for the most strongly associated SNP (rs11209032) in the British/North American populations (4). Interestingly, no association was established with rs11209032 in Portuguese or Spanish populations, which was strongly associated with AS in other Caucasian populations (4). Consistent with the Alberta

Table IV. Frequency of *IL-23R* minor allele frequencies in the Portuguese and Spanish cohorts.

Gene	SNP	Minor Allele	Portuguese Cohort				Spanish Cohort			
			Case MAF	Control MAF	OR	*p*-value	Case MAF	Control MAF	OR	*p*-value
IL23R	rs1004819	A	0.35	0.27	1.44 (1.13-1.84)	**4.9×10^{-3}**	0.32	0.29	1.21 (0.99-1.33)	0.076
IL23R	rs7517847	C	–	–	–		0.38	0.40	0.93 (0.76-1.13)	0.45
IL23R	rs10489629	C	0.43	0.45	0.93 (0.74-1.16)	0.51	0.42	0.46	0.92 (0.71-1.17)	0.50
IL23R	rs11465804	G	0.06	0.05	1.11 (0.68-1.78)	0.68	–	–	–	–
IL23R	rs11209026	A	0.04	0.04	0.98 (0,57-1.7)	0.95	0.03	0.07	0.46 (0.28-0.75)	**1×10^{-3}**
IL23R	rs1343151	A	0.33	0.34	0.93 (0.74-1.18)	0.56	0.29	0.38	0.68 (0.55-0.83)	**2×10^{-4}**
IL23R	rs10889677	A	0.28	0.36	1.41 (1.11-1.79)	**5.8×10^{-3}**	0.31	0.35	0.81 (0.65-0.99)	**3.9×10^{-2}**
IL23R	rs11209032	A	0.35	0.32	1.15 (0.91-1.45)	0.26	0.32	0.31	0.95 (0.77-1.16)	0.62
IL23R	rs1495965	C	0.45	0.45	0.98 (0.78-1.22)	0.84	0.44	0.43	0.96 (0.79-1.16)	0.69

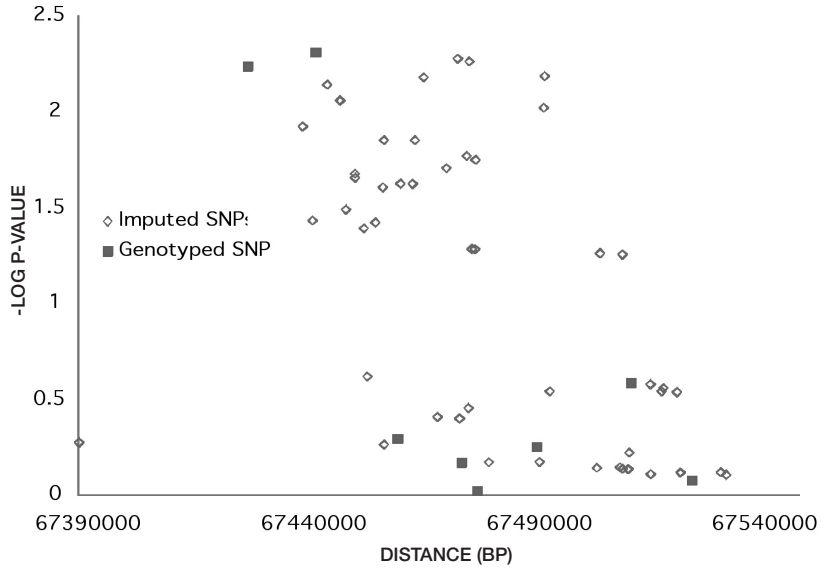

Fig. 2. Association results for imputed and directly genotyped SNPs in *IL23R*. Distances are in base pairs (bp) from the p-telomere. Association significance is reported as -$\log_{10}$(*p*-values).

Table V. Meta-analysis findings of combined Portuguese and Spanish studies of *IL23R*.

			Fixed effects		Random effects	
	Cochran Q *p*-value	I^2	OR	*p*-value	OR	*p*-value
rs1004819	0.28	0.16	1.3 (1.11-1.52)	$1.2x10^{-3}$	1.3 (1.10-1.55)	$2.7x10^{-3}$
rs10489629	0.61	0	0.89 (0.76-1.03)	0.12	0.89 (0.77-1.03)	0.11
rs11209026	0.017	0.83	0.59 (0.41-0.85)	$3.1x10^{-3}$	0.62 (0.26-1.5)	0.29
rs1343151	0.023	0.81	0.76 (0.65-0.89)	$4x10^{-4}$	0.77 (0.54-1.1)	0.16
rs10889677	0.0005	0.92	1.02 (0.87-1.19)	0.81	1.06 (0.62-1.83)	0.83
rs11209032	0.59	0	1.09 (0.94-1.27)	0.27	1.09(0.94-1.27)	0.27
rs1495965	0.68	0	1.01(0.87-1.17)	0.90	1.01 (0.88-1.17)	0.87

Canadian population, but in contrast to the British, United States, Spanish and Canadian (Toronto and Newfoundland) populations, the present study did not demonstrate any protective effect against AS for the Arg381Gln SNP (rs11209026) in the *IL23R* gene. This apparent lack of concordance may be due to the different ethnical backgrounds studied, or represent a type II error, given the modest power of the current study to detect association with this rare, protective, SNP. Given that significant association was seen with other SNPs in the Portuguese population, this suggests that rs11209026 and rs11209032 may not be the principal associated variants responsible for the *IL23R* association with AS, at least in the Iberian population. The imputed SNP data demonstrates that a broad region of *IL23R* is associated with AS in Portuguese. Association peaked in the 66kb region from rs10889667 to rs11465817, with a rapid reduction in strength of association outside of that region, suggesting that the primary associated variant(s) lies in that interval.

Differences are apparent between our Portuguese data, and the previously reported Spanish data, as reflected by the significant Cochran's Q statistic in the meta-analysis of *IL23R* SNPs. Whilst statistically significant, with one exception (rs10889617), the magnitude of the heterogeneity is small, reflected by the low values of the I^2 statistic. Therefore it is not entirely clear as to whether a fixed- or random- effects model should be applied, with the latter being more conservative and less powerful. Considering the random effects, just 2 SNPs, rs1004819, revealed association (Table IV). The findings in the Portuguese population for rs10889677 were very similar to those observed in both the discovery and replication set of the study by the WTCCC/TASC in the British and North Americans (4), and in marked contrast to those reported in the Spanish population (6). We observed a MAF in cases and controls of 34% and 28% for this SNP respectively, compared with 31% and 35% in the Spanish population. In the WTCCC/TASC study, the MAF for this SNP in cases and controls respectively were 36% and 31% in the discovery cohort, and 37% and 29% in the control cohort. Association was seen between rs10889677 and AS in the Spanish study (OR=0.81 with minor allele 'A', P=0.039), but curiously it was in the opposite direction to the two cohorts reported by the WTCCC/TASC study, and our own Portuguese study.

Numerous studies have demonstrated association of *IL23R* SNPs with susceptibility to Crohn's disease (CD) (17), as well as to psoriasis (18) and psoriatic arthritis (19-21). *IL23R* therefore seems to be a common susceptibility factor for the major seronegative diseases, at least partially explaining their co-occurrence. In contrast, *ERAP1* is not associated with inflammatory bowel disease, and it is unknown whether it is associated with psoriasis or psoriatic arthritis. Whether *IL23R* or *ERAP1* polymorphisms influence clinical manifestations of disease such as age of symptoms onset, disease activity or severity is unknown. In the current study no association was observed with these traits, but the study power was only adequate for large genetic effect sizes (>4% of the trait variance).

This study confirms the association of *ERAP1* variants with AS, with a similar magnitude of effect to that seen for *IL23R*, as assessed by the population attributable risk fraction. We are not aware of other papers that have replicated this finding in populations other than British or North Americans. The strongest associated in *ERAP1* was rs27044 (OR=1.29, 95% CI=1.02-1.63; *p*=0.032). In contrast with *IL23R*, the association of *ERAP1* seems to be confined to AS. No association was observed between *ERAP1* SNPs and either Crohn's disease or ulcerative colitis in the WTCCC/TASC study (4). Whether

ERAP1 SNPs are associated with psoriasis or psoriatic arthritis is unknown. No association was seen with the marker rs2303138 lying in *LNPEP*, providing further support to the hypothesis that at least a component of the association observed between this SNP and AS previously reported in British Caucasians is due to linkage disequilibrium with *ERAP1* polymorphisms.

The primary associated variant(s) in *ERAP1* remain uncertain. In this study, nominal association was seen between the SNPs rs26509 (96108436 from the p-telomere) and rs190298 (96191986 from the p-telomere), an interval of 84 kb. Very broad association was seen in the WTCCC/TASC study as well. Further fine-mapping and resequencing studies will be required to narrow the associated region and identify the key associated variant(s) to inform more targeted functional analysis of the mechanisms of involvement of this gene and AS.

Conclusions

These results show that *IL23R* and *ERAP1* genes are also associated with susceptibility to AS in the Portuguese population, and that they contribute a significant proportion of the population risk for this disease.

Authors' contributions

FPS, DL, HT, HGP, MAB and JCB participated in the design of the study. Experiments were performed by FPS, DL and MM. Statistical analysis was carried out by FPS, DME and MB. FPS, AFM, ES, PP, AR, MS, AB, FG and MC contributed by providing human samples. Analysis of data was carried out by FPS, DL, MM and MB. Intellectual contributions to the manuscript were provided by FPS, JEF, HGP, MAB and JCB. All authors read and approved the final manuscript.

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