Clinical and genetic characteristics of ankylosing spondylitis patients with peripheral arthritis at disease onset

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Abstract

Objective

The aim of this study was to assess the clinical and genetic characteristics associated with the presence of peripheral arthritis (PA) at disease onset in patients with ankylosing spondylitis (AS).

Methods

456 Spanish AS patients, diagnosed according to the modified New York Criteria, who had at least ten years of follow-up since initial disease onset were selected from the National Spondyloarthropathies Registry (REGISPONSER). 18.9% of AS patients initially presented PA. Clinical variables and 384 single nucleotide polymorphisms (SNPs) distributed in 190 genes were analysed. SNP genotyping was performed using the Illumina GoldenGate genotyping platform. Association tests for allele frequencies and for categorical clinical variables were performed by the χ^2 test and with the unpaired t-test for continuous variables. p-values of <0.05 were considered statistically significant.

Results

AS patients with PA showed an earlier age of disease onset (p=0.021), longer disease duration (p=0.020) and longer duration of AS symptoms from onset (p=0.034) than AS patients without PA. We found significant associations with the presence of PA at disease onset in 14 SNPs located in 10 genes: HLA-DQB2 (rs2857210 and rs9276615), HLA-DOB (rs2857151, rs2621332 and rs1383261), JAK2 (rs7857730), IL-23R (rs11209008 and rs10489630), CYP1B1 (rs1056836), NELL1 (rs8176786), KL (rs564481), and MEFV (rs224204), IL-2RB (rs743777) and IL-1A (rs1800587).

Conclusion

Both clinical and genetic factors are associated with the presence of PA at disease onset in Spanish AS patients. The results suggest that this subset of AS patients with PA at disease onset might have differentiation factors involved in disease pathogenesis.

Key words

ankylosing spondylitis, peripheral arthritis, single nucleotide polymorphism, JAK2, IL-23R, genetics

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease that mainly affects the axial skeleton and sacroiliac joints. However, a significant percentage of patients, ranging from 22 to 70% according to different studies, may also have peripheral arthritis (PA), generally oligoarticular with lower limb dominance (1-4). This manifestation usually occurs during the first years of the AS disease and it may precede spinal pain by several years. Furthermore, peripheral joint involvement commonly precedes axial symptoms in juvenile-onset spondylitis (JAS) (5).

At present there are very few studies that examine the clinical and genetic basis of the presence of PA in AS patients at disease onset. Several studies have reported that AS patients with PA show an earlier age of onset than those without PA (6-7). The study published by Lee *et al*., whose objective was to determine the clinical spectrum and manifestations of AS, showed that AS patients with PA had a higher frequency of enthesitis and trauma history (6) while the study of Singh *et al*. reported that extra-articular manifestations in the form of uveitis, dactylitis and enthesitis were more likely to be present in AS patients with PA (7). Chinese AS patients have a higher frequency of JAS with PA, as well as an earlier onset and more recurrent episodes of HLA-B27 associated acute anterior uveitis (AAU) (8). Furthermore, another study carried out in Chinese populace showed that JAS and adult-onset AS in patients had different presentations, whereby JAS more frequently presented peripheral enthesopathies and arthritides at disease onset or during any other time of the course of the disease (9).

Regarding genetic factors, there are few studies published (10-15) which investigate genetic associations in AS patients with PA. One shows an association with the G1181C polymorphism of the osteoprotegerin (OPG) gene (10). Other studies have found an association between PA and the HLA-DR4 allele (12) and the HLA-DR7 allele (13-14). Nevertheless, other AS studies found no effect of susceptibility of HLA-DR genes for PA (15).

These data suggest that the subset of AS patients with PA at disease onset might have different factors involved in disease pathogenesis. Hence, our objective was to assess different clinical and genetic features in AS patients with or without PA at disease onset.

Materials and methods

Study population

We carried out a cross-sectional study with 456 Spanish AS patients (108 female; 348 male). The patients, who fulfilled the modified New York criteria for AS diagnosis and had at least 10 years of follow-up from the first symptoms of the disease, were recruited from 25 hospitals participating in the Spanish National Registry of Spondyloarthropathies (REGISPONSER) (16). In each centre, there was a rheumatologist, previously trained in a 2-day session, responsible for the patient's assessment and data entry in a centralised system. Socio-demographic and clinical data were colleted through self-administered questionnaires to all patients and by physical examination of the patients at the inclusion visit. The following demographic and clinical characteristics of the patients were recovered from the Regisponser database: age, sex, family history of spondyloarthritis (SpA), HLA-B27 positivity, disease duration and duration of symptoms, age at disease onset, peripheral arthritis at the disease onset, clinical manifestations such as enthesitis, dactylitis, AAU, inflamamatory bowel disease (IBD) and psoriasis, Bath Ankylosing Spondylitis Functional Index (BASFI) score to measure functional impairment and the total Bath Ankylosing Spondylitis Radiology Index (BASRI-t) score to assess the disease structural damage. The clinical variable "peripheral arthritis" included arthritis of the upper and/or lower limbs and excluded arthritis of the hips and shoulders; "peripheral", in ankylosing spondylitis, is understood as large joints such as knees, elbows, ankles, carps as well as "small joints" of hands and feet (1, 17). The available data of treatments received by patients until the baseline visit, including non-steroidal anti-inflammatory drugs

(NSAIDs), corticoids, disease-modifying anti-rheumatic drugs (DMARDs) and biological therapies (infliximab, etanercept, adalimumab) were also recorded.

HLA-B27 typing and genotyping of SNPs

Genomic DNA from AS patients was isolated from saliva samples using the Oragene™ DNA Self-Collection Kit (DNA Genotek Inc., Ottawa, Canada), following the manufacturer's extraction protocol. Samples were tested for the presence of the HLA-B27 allele by conventional PCR using the primers described by Olerup *et al*. (18). A region of 236 bp of exon 8 of the *P53* gene was used as an internal control for the PCR performance (19).

We selected 384 SNPs distributed within 190 genes to be analysed for this study. SNPs from the metabolic pathways of two of the most important genes which are considered to be involved in AS were included: interleukin-23 receptor (*IL-23R)* and endoplasmic reticulum aminopeptidase-1 (*ERAP1*) genes, as these SNPs have been previously described as being associated with AS, as well as other spondyloarthropathies (psoriatic arthritis, juvenile idiopathic arthritis, reactive arthritis, undifferentiated arthritis, and inflammatory bowel disease-associated spondyloarthropathy), autoimmune diseases, and bone-related diseases. SNP genotyping was performed using the Illumina GoldenGate genotyping platform (Illumina, Inc., San Diego, CA, USA) (20).

Statistical analysis

Statistical analysis was performed with SPSS v. 15.0 (SPSS, Chicago, IL, USA) and SVS v. 7.3.1 (Golden Helix Inc., Bozeman, Montana, USA) software. All quantitative data were presented as mean and standard deviation (± SD) and all qualitative data as absolute frequencies and percentages. To standardise the measures of radiographic and functional severity, we adjusted BASRI-t and BASFI scores for duration of AS from the onset of the first symptoms of the disease (BASRI-t/duration) and (BASFI/duration). To assess the association between clinical variables and PA at disease onset, an unvaried analysis was performed using the chi-square (χ^2) test for categorical variables and the unpaired *t*-test for continuous variables. A test for deviation from Hardy-Weinberg equilibrium (HWE) was performed for each SNP using the Helix Tree™ software version 6.4.2 (Golden Helix Inc., Bozeman, Montana, USA). Pruning of the initial genotype dataset with default parameters was done to exclude SNPs with poor genotype cloud clustering, SNPs with a call-rate ≤85%, SNPs with severe deviation from HWE ($p<0.0001$), and samples with a call rate ≤85%, leading to the inclusion of 456 samples and 344 SNPs (21–24). An association test between allele and genotype frequencies and PA at disease onset was performed by the χ^2 test. The magnitude of association was expressed as an odds ratio (OR) with a 95% confidence interval (CI) (>1 indicates a susceptibility allele, and <1 indicates a protective allele). *P*-values were calculated using a single-value permutation test (1,000 permutations). *P*-values of <0.05 were considered statistically significant.

Ethical approval

This study was approved by the Ethics Committee of Puerta de Hierro Majadahonda University Hospital, Madrid, Spain. Each patient signed an informed consent form upon inclusion in REGISPONSER, in accordance with the fundamental principles set out in the Declaration of Human Rights of Helsinki.

Table I. Clinical and demographic characteristics of AS patients.

Characteristics	Values
Sex, Males/Females	348/108
Age, mean ± SD (years)	51 ± 11
Age at disease onset, mean ± SD (years)	26.06 ± 9.08
Disease duration*, mean ± SD (years)	16.2 ± 10.04
Duration of symptoms**, mean ± SD (years)	24.7 ± 10.11
Family history of SpA (%)	88 (19.3%)
HLA- B27 positive (%)	386 (84.6%)
BASFI, mean ± SD (years)	4.04 ± 2.80
BASFI/duration of AS, mean ± SD (years)	0.174 ± 0.13
BASRI-t, mean ± SD (years)	8.06 ± 4.18
BASRI-t/duration of AS, mean ± SD (years)	0.348 ± 0.19
Arthritis lower limbs	80 (17.5%)
Arthritis upper limbs	14 (3.1%)
Arthritis upper and/or lower limbs	86 (18.9%)

BASFI: Bath Ankylosing Spondylitis Functional Index; BASRI-t: total Bath Ankylosing Spondylitis Radiology Index; SD: standard deviation. *Disease duration refers to the period since diagnosis. **Duration of symptoms refers to the period since the onset of symptoms.

Results

Association between clinical variables and manifestation of PA

Baseline clinical and demographic characteristics are summarised in Table I. The average age of the 456 AS patients was 51±11 years. A total of 84.6% of AS patients were found to be HLA-B27 positive and 18.9% (86 patients) presented PA at disease onset. Among the patients with PA, the percentage of patients with arthritis in lower limbs was greater (17.5%, 80 patients) than those with arthritis in upper limbs (3.11%, 14 patients). The baseline clinical variables analysed: The age at disease onset, disease duration (from the time of diagnosis), and duration of AS symptoms (from the time of initial symptom onset) were found to be statistically significant. AS patients with PA presented a statistically significant earlier age of disease onset than AS patients without PA (24.02±9.18 *vs*. 26.53±9.00; $p=0.021$). Longer disease duration (18.65±10.16 *vs*. 15.76±9.95; $p=0.020$) and longer duration of AS symptoms (26.87±10.58 *vs*. 24.31±9.95; $p=0.034$) were also found to be associated with the presence of PA at disease onset in Spanish AS patients. A comparison of clinical and demographic characteristics is shown in Table II.

Table II. Clinical and demographic characteristics in AS patients with or without PA at disease onset.

Characteristic	AS w/o PA at onset (n=370)	AS with PA at onset (n=86)	*p*
Sex (males, %)	76.5 %	75.6 %	0.859
Age, mean ± SD (years)	50.84 ± 10.49	50.90 ± 10.71	0.962
Age at onset, mean ± SD (years)	26.53 ± 9.00	24.02 ± 9.18	0.021
Disease duration, mean ± SD (years)	15.76 ± 9.95	18.65 ± 10.16	0.020
Duration of symptoms, mean ± SD (years)	24.31 ± 9.95	26.87 ± 10.58	0.034
Family history of AS (%)	21.2 % (n=340)	20.8 % (n=77)	0.939
BASFI, mean ± SD (years)	4.05 ± 2.78	4.02 ± 2.90	0.926
BASFI/duration of AS, mean± SD (years)	0.176 ± 0.13510	0.1605 ± 0.13916	0.292
BASRI-t, media ± DS (years)	8.02 ± 4.11	8.20 ± 4.50	0.717
BASRI-t/duration of AS, mean± SD (years)	0.3532 ± 0.18727	0.3273 ± 0.20454	0.257
HLA-B27 positive (%)	84.8 %	84.9 %	0.989

AS: ankylosing spondylitis; PA: peripheral arthritis; BASFI: Bath Ankylosing Spondylitis Functional Index; BASRI-t: total Bath Ankylosing Spondylitis Radiology Index; SD: Standard deviation; HLA-B27: human leukocyte antigen B27. *p*-values of <0.05 were considered statistically significant.

Table III. Clinical manifestations that appear in the course of disease in AS patients with or without PA at disease onset.

Manifestation	n	AS w/o PA at onset	AS with PA at onset	*p*
IBD	449	17/365 (4.7%)	2/84 (2.4%)	0.350
Psoriasis	445	8/363 (2.2%)	4/82 (4.9%)	0.177
AAU	447	65/364 (17.9%)	22/83 (26.5%)	0.073
Enthesitis	456	125/370 (33.8%)	48/86 (55.8%)	$1.49X10^{-4}$
Dactylitis	456	19/370 (5.1%)	14/86 (16.3%)	$3.27X10^{-4}$

AS; ankylosing spondylitis; AAU, acute anterior uveitis; PA: peripheral arthritis; IBD: inflammatory bowel disease. *p*-values of <0.05 were considered statistically significant. *p*-values from 0.05 to 0.10 were considered borderline associations.

Table IV. Allelic variants associated with PA at disease onset in AS patients.

SNP	Gene	CR	Allele	OR (CI)	*p**
rs7857730	JAK2	9	A	1.98 (1.38-2.85)	0.001
rs2857210	HLA-DQB2	6	A	1.63 (1.15-2.32)	0.006
rs11209008	IL-23R	1	A	2.33 (1.17-4.67)	0.005
rs10489630	IL-23R	1	C	1.15 (1.08-2.12)	0.017
rs9276615	HLA-DQB2	6	G	1.52 (1.07-2.16)	0.016
rs2857151	HLA-DOB	6	A	1.52 (1.06-2.17)	0.011
rs1056836	CYP1B1	2	G	1.50 (1.06-2.18)	0.019
rs2621332	HLA-DOB	6	G	1.50 (1.05-2.15)	0.024
rs8176786	NELL1	11	G	7.25 (0.98-53.51)	0.019
rs564481	KL	13	A	1.46 (1.05-2.05)	0.022
rs224204	MEFV	16	G	1.47 (1.05-2.07)	0.021
rs743777	IL-2RB	22	G	1.46 (1.04-2.05)	0.023
rs1383261	HLA-DOB	6	G	1.49 (1.01-2.20)	0.042
rs1800587	IL-1A	2	G	1.50 (1.00-2.21)	0.043

SNP: single nucleotide polymorphism; CR: chromosome; OR: odds ratio; CI: confidence interval; JAK2: Janus Kinase 2; HLA-DQB2: human leukocyte antigen DQ beta 2 chain; IL-23R: interleukin-23 receptor; HLA-DOB: human leukocyte antigen DO beta chain; CYP1B1: cytochrome P450, family 1, subfamily B, polypeptide 1; NELL1: Nel-like protein 1; KL: klotho enzyme; MEFV: Mediterranean fever; IL-2RB: interleukin-2 receptor beta; IL-1A: interleukin-1 alpha.
*p**: *p*-values corrected using a single-value permutation test (1,000 permutations).
The magnitude of association was expressed as odds ratio (OR) with a 95% confidence interval (CI). Susceptibility alleles have showed (OR> 1). *p*-values of <0.05 were considered statistically significant.

Although disease duration and duration of AS symptoms were significantly longer in patients with PA, we did not find any statistically significant differences in functional impairment index (BASFI) scores or radiological progression index (BASRI-t) scores by adjusting for duration of AS symptoms (BASFI/duration and BASRI-t/duration, respectively). HLA-B27 was not found to be associated with the presence of PA at disease onset for AS patients.

There were no statistical differences between both groups of AS patients concerning gender and family history, although it is to be noted that the number of analysed patients in respect to family history was different in both groups because of missing values from the database.

Regarding the available data of received treatments, patients with PA at disease onset were treated in a higher percentage with corticosteroids (66.7% *vs*. 33.7%, *p*=0.002), DMARDs such as methotrexate (45.1% *vs*. 21.3%; $p=4.8 \times 10^{-4}$) and sulfasalazine (69.6% *vs*. 29.2%; $p=2.5 \times 10^{-8}$) and with the anti-TNF infliximab (37.0% *vs*. 22.3%, *p*=0.026) comparatively to AS patients without PA (data not shown).

Another difference assessed in both study groups was the prevalence of several clinical manifestations as enthesitis and dactylitis as well as some extra-articular clinical manifestations, including AAU, psoriasis and IBD. Enthesitis and dactylitis were significantly higher in AS patients with PA (55.8% *vs*. 33.8%; $p=1.49X10^{-4}$ and 16.3% *vs*. 5.1%, $p=3.27X10^{-4}$, respectively) than AS patients without PA. Although none of the extra-articular manifestations studied were significantly associated with the presence of PA in AS patients, there was a borderline association of AAU in the group of AS patients with PA (26.5% *vs*. 17.9%, *p*=0.073) (Table III).

Association between SNPs and manifestation of PA

In previous studies performed by our group, genetic factors had been demonstrated to cause an increased risk for AS susceptibility, as well as functional and radiographic severity and poor treatment response (23, 25-28). The aim of this study was to assess if previously studied polymorphisms could also be associated with the presence of PA at the onset of AS.

Data analysis showed a significant allelic association with the presence of PA in AS patients for 14 SNPs distrib-

Table V. Analysis of genotype and allele frequencies of several SNPs in different subgroups of AS patients with and without peripheral arthritis at disease onset, statistical significance and odds ratios (OR) values.

				AS+PA group					AS group								
		Allele		Genotypes			Alleles		Genotypes			Alleles		Alleles			Genotypes
SNP	N	1	2	11 n (%)	12 n (%)	22 n (%)	1 n (%)	2 n (%)	11 n (%)	12 n (%)	22 n (%)	1 n (%)	2 n (%)	OR (CI)	*p*	*p**	p
rs7857730	445	A	C*	36 (43.4)	44 (53.0)	3 (3.6)	116 (69.9)	50 (30.1)	109 (30.1)	172 (47.5)	81 (22.4)	390 (53.9)	334 (46.1)	0.50 (0.35-0.72)	0.00017	0.001	0,00024
rs2857210	448	A*	G	13 (15.3)	37 (43.5)	35 (41.2)	63 (37.1)	107 (62.9)	23 (6.3)	146 (40.2)	194 (53.4)	192 (26.4)	534 (73.6)	1.63 (1.15-2.32)	0.00577	0.006	0,01062
rs11209008	456	A*	G	0 (0.0)	13 (15.1)	73 (84.9)	13 (7.7)	159 (92.3)	0 (0.0)	25 (6.8)	345 (93.2)	25 (3.4)	715 (96.6)	2.33 (1.17-4.67)	0.01347	0.005	0,01152
rs10489630	454	A	C*	24 (27.9)	46 (53.5)	16 (18.6)	94 (54.6)	78 (45.3)	155 (42.1)	166 (45.1)	47 (12.8)	476 (64.7)	260 (35.3)	1.15 (1.08-2.12)	0.01435	0.017	0,04224
rs9276615	456	A	G*	36 (41.9)	38 (44.2)	12 (14.0)	110 (63.9)	62 (36.1)	196 (53.0)	148 (40.0)	26 (7.0)	540 (72.9)	200 (27.1)	1.52 (1.07-2.16)	0.01853	0.016	0,04952
rs2857151	444	A*	G	12 (14.5)	38 (45.8)	33 (39.8)	62 (37.3)	104 (62.7)	24 (6.6)	155 (42.9)	182 (50.4)	245 (47.1)	519 (67.9)	1.52 (1.06-2.17)	0.01906	0.011	0,03363
rs1056836	453	C*	G	7 (8.1)	45 (52.3)	34 (39.5)	59 (34.3)	113 (65.7)	74 (20.2)	174 (47.4)	119 (32.4)	322 (43.8)	412 (56.1)	0.66 (0.47-0.94)	0.02215	0.019	0,02983
rs2621332	414	A	G*	26 (36.9)	41 (51.9)	12 (15.2)	93 (58.9)	65 (41.1)	152 (45.4)	154 (46.0)	29 (8.7)	458 (68.3)	212 (31.7)	1.50 (1.05-2.15)	0.02284	0.024	0,06263
rs8176786	455	A*	G	0 (0.0)	1 (1.2)	85 (98.8)	1 (0.6)	171 (99.4)	1 (0.3)	28 (7.6)	340 (92.1)	30 (4.0)	708 (95.9)	0.13 (0.01-1.01)	0.02332	0.019	0,07865
rs564481	453	A*	G	21 (24.4)	39 (45.3)	26 (30.2)	81 (47.1)	91 (52.9)	54(14.7)	169 (46.0)	144 (39.2)	277 (37.8)	457 (62.2)	1.46 (1.05-2.05)	0.02389	0.024	0,06469
rs224204	455	A*	G	12 (14.1)	41 (48.2)	32 (37.6)	65 (38.2)	105 (61.8)	89 (24.1)	175 (47.3)	106 (28.6)	353 (47.7)	387 (52.3)	0.67 (0.48-0.95)	0.02550	0.019	0,08490
rs4639334	453	A	G*	11 (12.9)	10 (11.8)	64 (75.3)	32 (18.8)	13 (81.2)	66 (17.9)	67 (18.2)	235 (63.9)	199 (27.1)	537 (72.9)	0.62 (0.41-0.95)	0.02676	0.022	0,13089
rs743777	456	A	G*	35 (40.7)	31 (36.0)	20 (23.3)	101 (58.7)	71 (41.3)	165(44.6)	170(45.9)	35 (9.5)	500 (67.6)	240 (32.4)	1.46 (1.04-2.05)	0.02747	0.021	0,00166
rs3781202	436	A	G	21 (26.6)	30 (38.0)	28 (35.4)	72 (45.6)	86 (54.4)	125 (35.0)	139 (38.9)	93 (26.1)	389 (54.5)	325 (45.5)	1.42 (1.01-2.02)	0.04228	0.023	0,17862
rs1383261	446	A*	G	6 (7.1)	28 (33.3)	50 (59.5)	40 (23.8)	128 (76.2)	34 (9.4)	162(44.8)	166(45.9)	230 (31.8)	494 (68.2)	0.67 (0.45-0.98)	0.04309	0.042	0,07805
rs1800587	449	A	G*	3 (3.6)	32 (38.1)	49 (58.3)	38 (22.5)	130 (77.5)	35 (9.6)	152(41.6)	178(48.8)	222 (30.4)	508 (69.6)	0.66 (0.45-0.99)	0.04467	0.043	0,09496

AS: ankylosing spondylitis; PA: Peripheral arthritis; SNP: single nucleotide polymorphism; OR; odds ratio; CI: confidence interval; JAK2: Janus Kinase 2; HLA-DQB2: human leukocyte antigen DQ beta-chain; IL-23R: interleunkin 23 receptor; HLA-DOB: human leukocyte antigen DO beta-chain; CYP1B1: cytochrome P450, family 1, subfamily B, polypeptide 1; NELL1: Nel like 1; KL: klotho enzyme; MEFV: Mediterranean fever; IL-2RB: interleukin 2 receptor beta; IL-1A: interleukin 1 alpha.

*p**: *p*-values corrected using a single-value permutation test (1000 permutations).

The susceptibility or protective alleles for each SNP are highlighted with an asterisk (*); OR>1 values indicate susceptibility alleles and OR<1 values indicate protective alleles. *p*-values of <0.05 were considered statistically significant.

uted in 10 different genes (Table IV). The allele and genotype associations are shown in Table V. Five out of fourteen polymorphisms associated with the presence of PA are located in genes of the major histocompatibility complex (MHC): rs2857210 (*HLA-DQB2* gene, *p*=0.006), rs9276615 (*HLA-DQB2* gene, *p*=0.016), rs2857151 (*HLA-DOB* gene, *p*=0.011), rs2621332 (*HLA-DOB* gene, *p*=0.024), and rs1383261 (*HLA-DOB* gene, *p*=0.042). Three other SNPs are situated in genes related with the IL-23R) signalling pathway: rs7857730 Janus Kinase 2 - *JAK2* gene, *p*=0.001), rs11209008 (*IL-23R* gene, *p*=0.005), and rs10489630 (*IL-23R* gene, *p*=0.017). The remaining polymorphisms associated with the presence of PA at disease onset were: rs1056836 (*Cytochrome P450, Family 1, Subfamily B, Polypeptide 1- CYP1B1* gene, *p*=0.019), rs8176786 (*Nel like 1- NELL1* gene, *p*=0.019), rs564481 (*klotho enzyme- KL* gene, *p*=0.022), rs224204 (*Mediterranean fever - MEFV* gene, *p*=0.021), rs743777 (*interleukin-2 receptor beta- IL2RB* gene, *p*=0.023) and rs1800587 (*interleukin-1 alpha- IL1A* gene, *p*=0.043).

Discussion

In this study our main aim was to assess the genetic and clinical characteristics manifested by AS patients with PA at disease onset. Presently there are few studies which investigate distinctive variables in this subgroup of AS patients. Our results show that AS patients with PA had an earlier age at disease onset, longer disease duration and longer duration of AS symptoms from initial onset than AS patients without PA. These findings are consistent with previously published data which show that AS patients with PA had a higher prevalence of juvenile onset (7). Other studies indicate that JAS patients had more peripheral enthesopathies and arthritides at disease onset or during any other time of the course of the disease than patients with adult onset (9). Furthermore, Lee *et al*. showed that the age at onset in AS patients with PA was significantly earlier than patients without PA (6).

The analysis of several clinical manifestations as enthesitis and dactylitis as well as some associated extra-skeletal manifestations with the presence of PA at the time of disease onset done in our study revealed a significant association with enthesitis and dactylitis and a borderline association with AAU. Some studies have reported a higher frequency of enthesitis in AS patients with peripheral arthritis (6, 7). Singh *et al*., show that patients with PA have a significantly higher prevalence of AAU and other clinical manifestations like dactylitis, enthesitis and hip joint involvement (7). Data published by Maksymowych *et al*. support the finding that AS patients who developed PA were also more likely to develop AAU (29). Notably, AAU is the most common extra-articular feature of enthesitis-related arthritis, a subtype of juvenile idiopathic arthritis characterised by the presence of predominantly lower limb arthritis and enthesitis in boys above the age of 6 years old (30). The fact that this extra-articular clinical manifestation did not reach statistical significance in our cohort could be due to the sample size, a limitation of this study.

Of the few studies which analyse the association between genetic factors and PA in AS patients, none examine any genetic associations specifically in patients with PA at disease onset. We have found a significant allelic association between PA and 14 different SNPs

found in ten genes. The most significant association found was rs7857730, an intronic SNP located in the *JAK2* gene. Two SNPs of the IL-23R signalling pathway, located in the *IL-23R* gene, were found to have a significant association, as well as five SNPs located in the MHC class II region, specifically in the HLA-DQB2 and HLA-DOB loci.

In addition to HLA-B27 (31-32), non-B27 MHC class I genes (HLA-Bw60) (33), MHC class II genes (DRB1, DQA1, DQB1, DRB1, DPB1) and haplotypes (27, 34-36) have been described as having an associated risk of susceptibility, increased severity, poorer prognosis and/or concomitant diverse clinical manifestations in AS patients (37-41). Studies have found an association between PA and HLA-DR4 (12) and HLA-DR7 alleles (13-14). Accordingly, our data support the involvement of MHC class II genes in the manifestation of PA in AS patients. Although the objective of this work was not to study different genetic associations with the age of disease onset or with uveitis in AS patients, we were able to show that patients with PA had an earlier disease onset and they had a borderline association with AAU, both clinical manifestations being associated with MHC class II loci.

The polymorphism rs7857730 in the *JAK2* gene has been the most significant association we have found with the manifestation of PA. Besides the *JAK2* gene, our results found the involvement of two SNPs in the *IL-23R* gene, which participate jointly with JAK2 in the IL-23R signalling pathway. IL-23R polymorphisms were associated with AS for first time in the genome-wide association study presented by the Wellcome Trust Case Control Consortium in 2007 (42). Likewise, SNPs in the *IL-23R* gene have been associated with IBD (43), psoriasis (44), and more recently with psoriatic arthritis (45). IL-23 is a key cytokine essential for the proliferation and terminal differentiation of T helper 17 cells (T_h17), a cell population implicated in the pathogenesis of common inflammatory diseases, including: AS, psoriasis, rheumatoid arthritis, IBD and multiple sclerosis. The binding of IL-23 to its receptor, IL23R, triggers JAK2 activation which in turn causes phosphorylation of the signal transducer and activator of transcription 3 (STAT3). Polymorphisms in the *JAK2* and *STAT3* genes have been associated with susceptibility of IBD (46) while SNPs in *STAT3* have been associated with susceptibility of AS (47-49). Regarding the polymorphism rs7857730 of *JAK2*, previously published data showed a haplotype within the *JAK2* locus, including this SNP, was related to AS. The association of *JAK2* polymorphism rs7857730 in this study could demonstrate a more direct relation with the presence of PA. We hypothesised that this intronic polymorphism in *JAK2* may affect the alternative processing of the encoded protein, affecting signalling pathways in which this kinase participates. The risk allele A could result in an increase of protein expression that would favour signalling mediated by chemokines and interleukins leading to a greater proliferation and migration of pro-inflammatory cells like T_h17, and therefore trigger an inflammatory response which promotes the development of PA.

In summary, our study confirms that both, clinical and genetic factors play an important role in the development of PA in Spanish AS patients. An earlier age at disease onset, longer disease duration, longer duration of AS symptoms and, the presence of 14 SNPs located in 10 genes were significantly associated with the presence of PA at disease onset in these patients. Among the genetic factors, the *JAK2* gene which participates in the signalling pathway of several chemokines and interleukins, such as IL-23, was significantly associated with the development of PA. Nevertheless, further studies are needed to confirm all these genetics associations with PA at disease onset in additional cohorts of AS patients. Taken together, these results suggest that this subset of AS patients with PA at disease onset might have distinctive factors involved in the pathogenesis since the early development of symptomatology.

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