Joint Bone Spine 88 (2021) 105115

Contents lists available at ScienceDirect

Joint Bone Spine

journal homepage: www.elsevier.com

Original article

HLA association with the susceptibility to anti-synthetase syndrome

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suppl. Informations

ARTICLE INFO

Article history:
Accepted 13 November 2020
Available online 7 December 2020

Keywords:
Anti-synthetase syndrome
Anti-Jo-1 antibodies

ABSTRACT

Objective: To investigate the human leukocyte antigen (HLA) association with anti-synthetase syndrome (ASSD).
Methods: We conducted the largest immunogenetic *HLA-DRB1* and *HLA-B* study to date in a homogeneous cohort of 168 Caucasian patients with ASSD and 486 ethnically matched healthy controls by sequencing-based-typing.
Results: A statistically significant increase of *HLA-DRB1*03:01* and *HLA-B*08:01* alleles in patients with ASSD compared to healthy controls was disclosed (26.2% versus 12.2%, $P = 1.56E{-}09$, odds ratio–OR

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https://doi.org/10.1016/j.jbspin.2020.105115

HLA
*HLA-DRB1*03:01*
*HLA-B*08:01*
*HLA-DRB1*07:01*

[95% confidence interval–CI] = 2.54 [1.84–3.50] and 21.4% versus 5.5%, $P = 18.95E{-}18$, OR [95% CI] = 4.73 [3.18–7.05]; respectively). Additionally, *HLA-DRB1*07:01* allele was significantly decreased in patients with ASSD compared to controls (9.2% versus 17.5%, $P = 0.0003$, OR [95% CI] = 0.48 [0.31–0.72]). Moreover, a statistically significant increase of *HLA-DRB1*03:01* allele in anti-Jo-1 positive compared to anti-Jo-1 negative patients with ASSD was observed (31.8% versus 15.5%, $P = 0.001$, OR [95% CI] = 2.54 [1.39–4.81]). Similar findings were observed when HLA carrier frequencies were assessed. The *HLA-DRB1*03:01* association with anti-Jo-1 was unrelated to smoking history. No HLA differences in patients with ASSD stratified according to the presence/absence of the most representative non-anti-Jo-1 anti-synthetase autoantibodies (anti-PL-12 and anti-PL-7), arthritis, myositis or interstitial lung disease were observed.

Conclusions: Our results support the association of the HLA complex with the susceptibility to ASSD.

1. Introduction

Anti-synthetase syndrome (ASSD) is a connective tissue disease included in the idiopathic inflammatory myopathies (IIM) group, characterised essentially by the typical clinical triad of arthritis, myositis, and interstitial lung disease (ILD) [1–5]. Antiaminoacyl tRNA-synthetase antibodies (anti-ARS) are the main markers of ASSD, with anti-Jo-1 as the most frequent autoantibody identified in this condition [1,2,6,7]. Other anti-ARS, mainly anti-PL-12 and anti-PL-7, are less commonly found in ASSD [1,2,6,7]. The etiopathogenesis of ASSD remains unclear, although some pieces of evidence support the hypothesis that both genetic and environmental factors may play a relevant role [8].

The human leukocyte antigen (HLA) region encompasses a group of genes that encodes the most polymorphic human proteins, the class I and class II antigen-presenting molecules [9]. This region is crucial in controlling the immune response and is implicated in the pathogenesis of numerous diseases, mainly in those involving autoimmune phenomena [10,11]. Previous studies performed in heterogeneous groups of patients with different types of IIM or small series of patients with ASSD have described a potential association of HLA alleles with the development of anti-synthetase autoantibodies [12–19]. Amongst them, alleles of the Caucasian 8.1 ancestral haplotype (mainly *HLA-DRB1*03:01* and *HLA-B*08*:01) were suggested as the major HLA risk factors implicated in this process [12–19].

Taking all these considerations into account, we aimed to further establish the potential HLA implication in the etiopathogenesis of ASSD by conducting the largest immunogenetic study performed so far of *HLA-DRB1* and *HLA-B* associations in a homogeneous and well-defined cohort of Caucasian patients with ASSD.

2. Methods

2.1. Study population

A total of 168 unrelated Spanish patients of European ancestry diagnosed with ASSD were enrolled in this study. Centres involved in the recruitment of these patients included: Hospital Universitario Marqués de Valdecilla (Santander); Hospital Universitario Clínico San Cecilio (Granada); Hospital Clínico Universitario de Santiago (Santiago de Compostela); Complejo Asistencial Universitario de León (León); Hospital Universitario Araba (Vitoria); Hospital Clínico de Barcelona, Hospital Universitario de Bellvitge and Hospital Universitario Valle de Hebrón (Barcelona); and Hospital General Universitario Gregorio Marañón, Hospital Universitario Fundación Jiménez Díaz, Hospital Universitario La Paz and Hospital Universitario de la Princesa (Madrid).

Patients with a positive anti-synthetase antibody test on at least two occasions, along with one or more findings of the typical clinical triad (arthritis, myositis and/or ILD), were recruited. Anti-synthetase autoantibodies were detected by the commercially available myositis immunoblot kit 'Euroline Autoimmune Inflammatory Myopathies 16 Ag kit' (Euroimmun, Luebeck, Germany) according to the manufacturer's instructions. This kit detects, amongst others, antibodies against the following tRNAs: Jo-1 (histidyl-tRNA synthetase), PL-12 (alanyl-tRNA synthetase), PL-7 (threonyl-tRNA synthetase), EJ (glycyl-tRNA synthetase) and OJ (isoleucyl-tRNA synthetase). In some cases, anti-Jo-1 antibodies were also assessed with commercially available ENA (extractable nuclear antigen) screen tests. Arthritis occurrence and its presentation pattern were clinically assessed by the treating rheumatologist (referent physician); myositis was defined by muscle enzyme elevation (creatinine phosphokinase and/or aldolase) and the presence of typical electromyography alterations and/or compatible muscle biopsy findings and/or compatible muscle magnetic resonance; ILD was defined instrumentally by a restrictive pulmonary function test pattern [forced vital capacity (FVC) $\leq$ 80%, forced expiratory volume in one second (FEV1)/FVC $\geq$ 70%], and/or diffusing capacity of the lung for carbon monoxide (DLCO) < 80% and interstitial changes on high-resolution computed tomography of the lungs [1,4,7,20–22]. Information on smoking history was also collected. With respect to this, 69.2% of our patients were female, median age at disease onset was 48.0 [38.0–60.0] years and 42.6% were ever-smokers. The specificities of the immunological tests in our patients were anti-Jo-1 in 65.5% ($n = 110$), anti-PL-12 in 13.7% ($n = 23$), anti-PL-7 in 13.1% ($n = 22$), anti-EJ in 1.8% ($n = 3$) and anti-OJ in 0.6% ($n = 1$). Amongst the other specificities disclosed in the immunoblot, Ro-52 was the most common, found in 39.3% of our patients ($n = 66$). In addition, arthritis, myositis and ILD were present in 61.3%, 71.4% and 91.1% of our patients, respectively. The occurrence of accompanying features, including fever, Raynaud's phenomenon and mechanic's hands were also assessed. Fever was defined as a body temperature $\geq$ 38 °C for more than 10 days with no evidence of an alternative cause. Raynaud's phenomenon was determined as the occurrence of transient finger ischemia after cold exposure. Mechanic's hands were defined as the occurrence of a thickened, hyperkeratotic, and fissured aspect of the radial sides of the fingers of the hands, in the absence of other causes [4,22,23]. Patients with other types of IIM were excluded from our study. Complete information on demographic and clinical features of the patients with ASSD enrolled in this study is displayed in Table S1 [Appendix A; See the supplementary material associated with this article online].

The control population included 486 ethnically matched unaffected individuals with no history of autoimmune disease, comprised by blood donors from Hospital Universitario Marqués de Valdecilla (Santander) and National DNA Bank Repository (Salamanca).

All patients and healthy controls gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki and all

Table 1
HLA-DRB1 allele frequencies in patients with ASSD and healthy controls.

HLA-DRB1		Patients with ASSD ($2n = 336$)	Healthy controls ($2n = 972$)	OR [95% CI]
*DRB1*01*	01:01	5.4 (18)	6.0 (58)	0.89 [0.49–1.56]
	01:02	3.9 (13)	5.6 (54)	0.68 [0.34–1.29]
	01:03	0.6 (2)	0.6 (6)	0.96 [0.09–5.42]
DRB1*03	**03:01**	**26.2 (88)**[a]	**12.2 (119)**[a]	**2.54 [1.84–3.50]**[a]
*DRB1*04*	04:01	1.5 (5)	2.7 (26)	0.55 [0.16–1.47]
	04:02	2.1 (7)	2.0 (19)	1.07 [0.38–2.68]
	04:03	1.5 (5)	0.5 (5)	2.92 [0.67–12.77]
	04:04	2.7 (9)	3.5 (34)	0.76 [0.32–1.64]
	04:05	4.8 (16)	2.4 (23)	2.06 [1.00–4.13]
	04:07	0.6 (2)	0.6 (6)	0.96 [0.09–5.42]
	04:08	0	0.2 (2)	0.00 [0.00–5.57]
DRB1*07	**07:01**	**9.2 (31)**[a]	**17.5 (170)**[a]	**0.48 [0.31–0.72]**[a]
*DRB1*08*	08:01	4.2 (14)	2.0 (19)	2.18 [1.00–4.64]
	08:02	0	0.1 (1)	–
	08:03	1.2 (4)	0.4 (4)	2.92 [0.54–15.73]
*DRB1*09*	09:01	0	0.9 (9)	0.00 [0.00–1.23]
*DRB1*10*	10:01	0	2.3 (22)	0.00 [0.00–0.49]
*DRB1*11*	11:01	6.3 (21)	6.9 (67)	0.90 [0.52–1.52]
	11:02	1.2 (4)	1.5 (15)	0.77 [0.18–2.43]
	11:03	0	1.1 (11)	0.00 [0.00–1.00]
	11:04	3.0 (10)	5.6 (54)	0.52 [0.23–1.05]
*DRB1*12*	12:01	0.3 (1)	0.7 (7)	0.41 [0.009–3.22]
*DRB1*13*	13:01	6.3 (21)	6.7 (65)	0.93 [0.53–1.57]
	13:02	2.4 (8)	3.3 (32)	0.72 [0.28–1.61]
	13:03	1.2 (4)	2.2 (21)	0.55 [0.14–1.63]
*DRB1*14*	14:01	1.5 (5)	1.9 (18)	0.80 [0.23–2.26]
	14:04	0.9 (3)	0.9 (9)	0.96 [0.17–3.89]
*DRB1*15*	15:01	6.8 (23)	6.1 (59)	1.14 [0.66–1.91]
	15:02	1.5 (5)	1.3 (13)	1.11 [0.31–3.37]
*DRB1*16*	16:01	3.3 (11)	2.1 (20)	1.61 [0.69–3.57]
	16:02	0.3 (1)	0.2 (2)	1.45 [0.02–27.88]

HLA: human leukocyte antigen; ASSD: anti-synthetase syndrome; OR: odds ratio; CI: confidence interval. Values are presented as percentages (number of individuals). Results that remained statistically significant after Bonferroni adjustment are highlighted in bold.
[a] $P < 0.001$

experimental protocols were approved by the Ethics Committees of clinical research of the corresponding centres (Hospital Universitario Marqués de Valdecilla, Hospital Universitario Clínico San Cecilio, Hospital Clínico Universitario de Santiago, Complejo Asistencial Universitario de León, Hospital Universitario Araba, Hospital Clínico de Barcelona, Hospital Universitario de Bellvitge, Hospital Universitario Valle de Hebrón, Hospital General Universitario Gregorio Marañón, Hospital Universitario Fundación Jiménez Díaz, Hospital Universitario La Paz and Hospital Universitario de la Princesa).

2.2. HLA-DRB1 and HLA-B Typing

High-molecular-weight genomic DNA was extracted from whole blood using the Maxwell 16 Blood DNA Purification Kit (Promega Biotech Ibérica, S.L., Spain) according to the manufacturer's instructions. All DNA samples were stored at −20 °C until the HLA analysis.

DNA-based *HLA-DRB1* and *HLA-B* typing were performed using the high-resolution typing through a sequencing-based typing (SBT) technique with the SBTexcellerator Kit and analysed with the SBTengine-SBT HLA typing software (GenDx, Utrecht, The Netherlands).

2.3. Statistical Analysis

HLA-DRB1 and *HLA-B* allele and carrier frequencies were calculated by direct counting. Comparisons between *HLA-DRB1* and *HLA-B* allele and carrier frequencies of patients with ASSD and healthy controls as well as patients with ASSD stratified according to antibody-specificity (presence/absence of anti-Jo-1, anti-PL-12 and anti-PL-7) and the most relevant clinical features of the disease (presence/absence of arthritis, myositis or ILD) were performed using the two-tailed chi-square test or the Fisher's exact test when necessary (expected values < 5). The strength of association was estimated using odds ratio (OR) and 95% confidence interval (CI). Results were subjected to Bonferroni adjustment for multiple comparisons. After this adjustment, *P*-values < 0.05 were considered statistically significant.

Dependency of associations between HLA susceptibility signals was determined by a conditional logistic regression analysis in which the allelic dosage of the classic alleles associated with ASSD in our analysis was added as a covariate.

Interaction of HLA and smoking for the development of anti-Jo-1 antibodies was analysed by chi-square test and the strength of association was estimated using OR and 95% CI.

All analyses were performed using the software Plink (version 1.07) [24]. Since Plink introduces a simple and efficient binary encoding for bi-allelic markers, HLA dataset was converted into the standard Plink data input before the statistical analysis.

3. Results

Firstly, *HLA-DRB1* allele and carrier frequencies were compared between patients with ASSD and healthy controls. Table 1 shows the *HLA-DRB1* allele differences between these two groups. Of note, the frequency of *HLA-DRB1*03:01* allele was significantly increased in patients with ASSD when compared to healthy controls (26.2% versus 12.2%, $P = 1.56E\text{-}09$, OR [95% CI] = 2.54 [1.84–3.50]) (Table 1). A statistically significant decrease of *HLA-DRB1*07:01* allele in patients with ASSD compared to healthy controls was also found (9.2% versus 17.5%, $P = 0.0003$, OR [95% CI] = 0.48 [0.31–0.72]) (Table 1). Similar results were disclosed when *HLA-DRB1* carrier frequencies were compared between patients with ASSD and

Table 2
HLA-B allele frequencies in patients with ASSD and healthy controls.

HLA-B		Patients with ASSD ($2n = 336$)	Healthy controls ($2n = 972$)	OR [95% CI]
*HLA-B*07*	07:02	4.2 (14)	7.0 (68)	0.58 [0.30–1.06]
	07:05	0.9 (3)	1.1 (11)	0.79 [0.14–3.00]
HLA-B*08	**08:01**	**21.4 (72)**[a]	**5.5 (53)**[a]	**4.73 [3.18–7.05]**[a]
*HLA-B*13*	13:02	1.8 (6)	1.7 (17)	1.02 [0.33–2.74]
*HLA-B*14*	14:01	1.8 (6)	1.9 (18)	0.96 [0.31–2.56]
	14:02	4.2 (14)	5.3 (52)	0.77 [0.39–1.43]
*HLA-B*15*	15:01	3.6 (12)	2.0 (19)	1.86 [0.81–4.08]
	15:03	0.3 (1)	0.6 (6)	0.48 [0.01–4.00]
	15:16	0	0.2 (2)	0.00 [0.00–5.57]
	15:17	1.2 (4)	0.4 (4)	2.91 [0.54–15.73]
	15:18	0.6 (2)	0.5 (5)	1.16 [0.11–7.11]
*HLA-B*18*	18:01	6.0 (20)	8.8 (86)	0.65 [0.37–1.09]
*HLA-B*27*	27:05	1.8 (6)	2.6 (25)	0.69 [0.23–1.74]
*HLA-B*35*	35:01	4.5 (15)	4.1 (40)	1.09 [0.55–2.05]
	35:02	0.9 (3)	2.1 (20)	0.43 [0.08–1.46]
	35:03	1.8 (6)	2.3 (22)	0.79 [0.26–2.02]
	35:08	0.6 (2)	2.3 (22)	0.26 [0.03–1.06]
*HLA-B*37*	37:01	0.9 (3)	1.0 (10)	0.87 [0.15–3.39]
*HLA-B*38*	38:01	1.8 (6)	4.5 (44)	0.38 [0.13–0.91]
*HLA-B*39*	39:01	2.1 (7)	1.1 (11)	1.86 [0.61–5.30]
	39:06	0.3 (1)	1.0 (10)	0.29 [0.007–2.03]
*HLA-B*40*	40:01	2.1 (7)	2.2 (21)	0.96 [0.34–2.38]
	40:02	1.2 (4)	0.7 (7)	1.66 [0.35–6.58]
	40:06	0	0.2 (2)	0.00 [0.00–5.57]
*HLA-B*41*	41:01	0.9 (3)	1.6 (16)	0.54 [0.10–1.90]
	41:02	0	0.2 (2)	0.00 [0.00–5.57]
*HLA-B*44*	44:02	3.9 (13)	7.1 (69)	0.53 [0.26–0.98]
	44:03	4.5 (15)	8.8 (86)	0.48 [0.25–0.85]
	44:04	0.3 (1)	0.1 (1)	2.90 [0.04–227.74]
	44:05	0	0.2 (2)	0.00 [0.00–5.57]
*HLA-B*45*	45:01	1.2 (4)	1.5 (15)	0.77 [0.18–2.43]
*HLA-B*47*	47:01	0.9 (3)	0.3 (3)	2.91 [0.39–21.81]
*HLA-B*49*	49:01	3.3 (11)	3.6 (35)	0.91 [0.41–1.85]
*HLA-B*50*	50:01	3.0 (10)	2.1 (20)	1.46 [0.60–3.31]
*HLA-B*51*	51:01	5.7 (19)	6.6 (64)	0.85 [0.47–1.46]
*HLA-B*52*	52:01	1.2 (4)	1.6 (16)	0.72 [0.17–2.25]
*HLA-B*53*	53:01	0.9 (3)	1.1 (11)	0.79 [0.14–3.00]
*HLA-B*55*	55:01	1.5 (5)	1.2 (12)	1.21 [0.33–3.72]
*HLA-B*56*	56:01	0.6 (2)	0.3 (3)	1.93 [0.16–16.95]
*HLA-B*57*	57:01	1.2 (4)	2.6 (25)	0.46 [0.11–1.34]
*HLA-B*58*	58:01	0.9 (3)	1.0 (10)	0.87 [0.15–3.39]
*HLA-B*73*	73:01	0.3 (1)	0.1 (1)	2.90 [0.04–227.74]

HLA: human leukocyte antigen; ASSD: anti-synthetase syndrome; OR: odds ratio; CI: confidence interval. Values are presented as percentages (number of individuals). Results that remained statistically significant after Bonferroni adjustment are highlighted in bold.

[a] $P < 0.001$

healthy controls. Accordingly, a statistically significant increase of *HLA-DRB1*03:01* and a statistically significant decrease of *HLA-DRB1*07:01* carriers were observed when patients with ASSD were compared to healthy controls (50.6% versus 22.6%, $P = 8.51E-12$, OR [95% CI] = 3.50 [2.38–5.15] and 17.9% versus 31.7%, $P = 0.0006$, OR [95% CI] = 0.47 [0.29–0.74]; respectively) (Appendix A, Table S2). These associations remained statistically significant after Bonferroni correction. Regarding *HLA-DRB1*03:01/HLA-DRB1*07:01* heterozygotes, no statistically significant differences were found when patients with ASSD were compared to healthy controls (5.6% versus 4.9%, respectively, $P = 0.61$, OR [95% CI] = 1.22 [0.51–2.71]).

Secondly, *HLA-B* allele and carrier frequencies were compared between patients with ASSD and healthy controls (Table 2 and Appendix A – Table S3). The strongest allele association was seen for *HLA-B*08:01*, which was significantly increased in patients with ASSD compared to healthy controls (21.4% versus 5.5%, $P = 18.95E-18$, OR [95% CI] = 4.73 [3.18–7.05]) (Table 2). Also, a statistically significant increase of *HLA-B*08:01* carriers in patients with ASSD compared to healthy controls was disclosed (42.3% versus 10.9%, $P = 3.96E-19$, OR [95% CI] = 5.98 [3.85–9.29]) (Appendix A, Table S3). Again, these associations remained statistically significant after Bonferroni correction.

In a further step, we also assessed the potential dependency between the three HLA susceptibility signals mentioned above. Interestingly, the association of *HLA-DRB1*03:01* with ASSD susceptibility remained statistically significant even after conditioning by *HLA-DRB1*07:01* and *HLA-B*08:01*, although a decrease on the *P*-value was observed when *HLA-DRB1*03:01* association was conditioned on *HLA-B*08:01* (Table 3). This was also the case when the association with *HLA-DRB1*07:01,* after conditioning by *HLA-DRB1*03:01* and *HLA-B*08:01,* and the association with *HLA-B*08:01,* after conditioning by *HLA-DRB1*03:01* and *HLA-DRB1*07:01,* were assessed (Table 3).

Moreover, *HLA-DRB1 a*nd *HLA-B* allele and carrier frequencies were compared between patients with ASSD stratified according to the presence/absence of anti-Jo-1, anti-PL-12 and anti-PL-7 antibodies and specific clinical features of the disease. In this regard, a statistically significant increase of *HLA-DRB1*03:01* allele and carriers in anti-Jo-1 positive patients with ASSD compared to anti-Jo-1 negative ones was detected (31.8% versus 15.5%, $P = 0.001$, OR [95% CI] = 2.54 [1.39–4.81] and 60.9% versus 31.0%, $P = 0.0002$, OR [95% CI] = 3.46 [1.68–7.24]) (Table 4 and Appendix A – Table S4). This association also remained statistically significant after Bonferroni correction. However, no statistically significant *HLA-B* allele and

Table 3
Analysis for the dependency of the three HLA signals associated with the susceptibility to ASSD.

ASSD versus healthy controls				
Alleles	*P*-values	*P*-values conditioned on *HLA-DRB1*03:01*	*P*-values conditioned on *HLA-DRB1*07:01*	*P*-values conditioned on *HLA-B*08:01*
*HLA-DRB1*03:01*	**2.48E-06**	–	**6.34E-05**	**0.026**
*HLA-DRB1*07:01*	**0.0003**	**0.009**	–	**0.004**
*HLA-B*08:01*	**1.40E-13**	**7.13E-07**	**1.33E-12**	–

HLA: human leukocyte antigen; ASSD: anti-synthetase syndrome. *P*-values before and after conditioning calculated by logistic regression. Statistically significant results are highlighted in bold.

Table 4
HLA-DRB1 allele frequencies in patients with ASSD stratified according to the presence/absence of anti-Jo1 antibodies.

HLA-DRB1		ASSD anti-Jo-1 positive ($2n = 220$)	ASSD anti-Jo-1 negative ($2n = 116$)	OR [95% CI]
*DRB1*01*	01:01	5.5 (12)	5.2 (6)	1.06 [0.36–3.53]
	01:02	3.6 (8)	4.3 (5)	0.84 [0.24–3.34]
	01:03	0.9 (2)	0	–
DRB1*03	**03:01**	**31.8 (70)**[a]	**15.5 (18)**[a]	**2.54 [1.39–4.81]**[a]
*DRB1*04*	04:01	1.4 (3)	1.7 (2)	0.79 [0.09–9.57]
	04:02	1.4 (3)	3.4 (4)	0.39 [0.06–2.34]
	04:03	1.4 (3)	1.7 (2)	0.79 [0.09–9.57]
	04:04	1.4 (3)	5.2 (6)	0.25 [0.04–1.22]
	04:05	4.5 (10)	5.2 (6)	0.87 [0.28–3.00]
	04:07	0.9 (2)	0	–
	04:08	0	0	–
*DRB1*07*	07:01	6.8 (15)	13.8 (16)	0.46 [0.20–1.03]
*DRB1*08*	08:01	5.0 (11)	2.6 (3)	1.98 [0.51–11.27]
	08:02	0	0	–
	08:03	0.9 (2)	1.7 (2)	0.52 [0.04–7.32]
*DRB1*09*	09:01	0	0	–
*DRB1*10*	10:01	0	0	–
*DRB1*11*	11:01	4.5 (10)	9.5 (11)	0.45 [0.17–1.22]
	11:02	0.5 (1)	2.6 (3)	0.17 [0.003–2.18]
	11:03	0	0	–
	11:04	2.3 (5)	4.3 (5)	0.52 [0.12–2.30]
*DRB1*12*	12:01	0.5 (1)	0	–
*DRB1*13*	13:01	8.2 (18)	2.6 (3)	3.35 [0.95–18.12]
	13:02	2.3 (5)	2.6 (3)	0.88 [0.17–5.74]
	13:03	0.9 (2)	1.7 (2)	0.52 [0.04–7.32]
*DRB1*14*	14:01	1.4 (3)	1.7 (2)	0.79 [0.09–9.57]
	14:04	1.4 (3)	0	–
*DRB1*15*	15:01	6.4 (14)	7.8 (9)	0.81 [0.31–2.19]
	15:02	0.9 (2)	2.6 (3)	0.35 [0.03–3.07]
*DRB1*16*	16:01	3.2 (7)	3.4 (4)	0.92 [0.23–4.38]
	16:02	0.5 (1)	0	–

HLA: human leukocyte antigen; ASSD: anti-synthetase syndrome; OR: odds ratio; CI: confidence interval. Results that remained statistically significant after Bonferroni adjustment are highlighted in bold. Values are presented as percentages (number of individuals).
[a] $P < 0.01$

carrier differences in patients with ASSD stratified according to the presence/absence of anti-Jo-1 antibodies were found after the adjustment by Bonferroni (Appendix A, Tables S5 and S6). This was also the case when *HLA-DRB1* and *HLA-B* allele and carrier frequencies were compared in patients with ASSD stratified according to the presence/absence of anti-PL-12 and anti-PL-7 antibodies (data not shown).

Since an interrelationship between *HLA-DRB1*03* and smoking was postulated to promote anti-Jo-1 production in IIM [25], we analysed the potential interaction of *HLA-DRB1*03:01* and smoking for the development of anti-Jo-1 antibodies in our patients with ASSD. However, the frequency of anti-Jo-1 antibodies was similar in *HLA-DRB1*03:01*-positive non-smokers versus ever-smokers (83.3% versus 80.0%, $P = 0.72$, OR [95% CI] = 1.25 [0.30–4.95]) (Appendix A, Table S7).

No *HLA-DRB1* or *HLA-B* differences in patients with ASSD stratified according to the presence/absence of arthritis, myositis or ILD after Bonferroni correction were detected (data not shown).

4. Discussion

Autoimmune inflammatory diseases are conditions characterised by common pathogenic traits and overlap in genetic risk [26–28]. In this regard, the HLA region has been identified as the main genetic factor underlying immune-mediated diseases [29,30].

To the best of our knowledge, we report the largest immunogenetic study of *HLA-DRB1* and *HLA-B* associations performed in a homogeneous and well-defined cohort of Caucasian patients with ASSD. Our results revealed three HLA signals implicated in the susceptibility to ASSD. In particular, the *HLA-DRB1*03:01* and *HLA-B1*08:01* alleles were identified as predisposition markers of ASSD whereas *HLA-DRB1*07:01* had a protective effect against this condition. We also disclosed an effect of the HLA on anti-Jo-1-positive specificity, pointing to *HLA-DRB1*03:01* as a risk allele for the development of these autoantibodies, irrespective of smoking status.

Alleles comprising the Caucasian 8.1 ancestral haplotype (mainly *HLA-DRB1*03:01* and *HLA-B1*08:01*) had been previously

suggested as the major HLA risk factor related to ASSD in heterogeneous cohorts of patients with IIM or small series of patients with ASSD [12–19]. The results derived from our large, homogeneous, and well-defined cohort of ASSD patients are in keeping with those described in previous reports, supporting the role of these alleles in the etiopathogenesis of ASSD. In this regard, the 8.1 ancestral haplotype is a common tightly conserved multigene haplotype in Caucasians [31] that influences several aspects of the immune response [32]. The constituent alleles of this haplotype are implicated in the development of several highly prevalent autoimmune diseases, such as systemic lupus erythematosus [33,34], systemic sclerosis [35], Sjögren syndrome [32] and myasthemia gravis [36,37]. Accordingly, our findings provide evidence for a shared genetic background in ASSD and other immunomediated diseases, primarily those related to autoantibody production.

An influence of *HLA-DRB1*07:01*, as a protective factor against the susceptibility to ASSD, was disclosed in our study. This is in agreement with a previous report suggesting a potential association of HLA alleles unrelated to the Caucasian 8.1 ancestral haplotype with ASSD [12].

Smoking has been linked to disease susceptibility and severity, including the development of ILD, in some autoimmune diseases, such as rheumatoid arthritis (RA) [38]. However, we did not observe the previously suggested interaction between smoking and *HLA-DRB1*03* for anti-Jo-1 development [25]. ILD in the context of RA has been associated with the *MUC5B* rs35705950 genetic variant [39]. In contrast, a previous study of our group did not show association of *MUC5B* rs35705950 with ILD in patients with ASSD [40]. These results along with data showing a different *HLA-DRB1* association in RA-ILD (mostly linked to *HLA-DRB1*04*) and ASSD suggest that the mechanisms leading to ILD in RA and ASSD may be different.

The differential diagnosis of ASSD is frequently challenging since the clinical presentation of the disease is diverse and often non-specific [2]. Consequently, patients with ASSD are frequently under-diagnosed. Our results are of potential clinical relevance since the characterisation of HLA together with the presence of specific clinical characteristics can help the clinician raise a flag for the suspicion of ASSD.

In conclusion, our results support the association of the HLA with the susceptibility to ASSD.

Funding

This study was partially supported by grants from the Foundation for Research in Rheumatology (FOREUM); SR-M is supported by funds of the RETICS Program [grant number RD16/0012/0009] from the 'Instituto de Salud Carlos III(ISCIII), co-funded by the European Regional Development Fund (ERDF); BA-M is a recipient of a 'López Albo' Post-Residency Programme funded by Servicio Cántabro de Salud; VP-C is supported by a pre-doctoral grant from IDIVAL [grant number PREVAL 18/01]; LL-G is supported by funds of ISCIII, co-funded by ERDF [grant number PI18/00042]; OG is beneficiary of a grant funded by Xunta de Galicia, Consellería de Educación, Universidade e Formación Profesional and Consellería de Economía, Emprego e Industria (GAIN), GPC IN607B2019/10; EAR is partially supported by Versus Arthritis [grant number 20719] and by Scleroderma and Raynaud's UK [grant number BR11]; RL-M is a recipient of a Miguel Servet type I programme fellowship from the ISCIII, co-funded by the European Social Fund (ESF, 'Investing in your future') [grant number CP16/00033].

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgments

We are indebted to the patients and healthy controls for their essential collaboration to this study. We also thank the National DNA Bank Repository (Salamanca) for supplying part of the control samples.

Appendix A. Supplementary data

Supplementary data (Tables S1–S7) associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jbspin.2020.105115.

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