



Original article

HLA association with the susceptibility to anti-synthetase syndrome

Sara Remuzgo-Martínez^{a,1}, Belén Atienza-Mateo^{a,b,1}, J. Gonzalo Ocejo-Vinyals^{c,1}, Verónica Pulito-Cueto^a, Diana Prieto-Peña^a, Fernanda Genre^a, Ana Marquez^{d,e}, Javier Llorca^f, Víctor M. Mora Cuesta^a, David Iturbe Fernández^a, Laura Riesco^c, Norberto Ortego-Centeno^e, Nair Pérez Gómez^g, Antonio Mera^g, Julia Martínez-Barrio^h, Francisco Javier López-Longo^h, Leticia Lera-Gómez^a, Clara Morianoⁱ, Elvira Díezⁱ, Eva Tomero^j, Jaime Calvo-Alén^k, Fredeswinda Romero-Bueno^l, Olga Sanchez-Pernaute^l, Laura Nuño^m, Gema Bonilla^m, Ignacio Grafiaⁿ, Sergio Prieto-Gonzálezⁿ, Javier Narvaez^o, Ernesto Trallero-Araguas^p, Albert Selva-O'Callaghan^p, Oreste Gualillo^q, Javier Martín^d, Lorenzo Cavagna^r, Santos Castañeda^j, José M. Cifrian^{a,s}, Elisabetta A. Renzoni^t, Raquel López-Mejías^{a,2,*}, Miguel A. González-Gay^{a,s,u,2,**}

^a Research group on genetic epidemiology and atherosclerosis in systemic diseases and in metabolic bone diseases of the musculoskeletal system, IDIVAL, Hospital Universitario Marqués de Valdecilla, Santander, Spain

^b 'López Albo' post-residency programme, Hospital Universitario Marqués de Valdecilla, Santander, Spain

^c Department of Immunology, Hospital Universitario Marqués de Valdecilla, Santander, Spain

^d Instituto de Parasitología y Biomedicina 'López-Neyra', CSIC, PTS Granada, Granada, Spain

^e Systemic Autoimmune Disease Unit, Hospital Universitario Clínico San Cecilio, Instituto de Investigación Biosanitaria ibs. GRANADA, Granada, Spain

^f Department of Epidemiology and Computational Biology, School of Medicine, University of Cantabria, and CIBER Epidemiología y Salud Pública (CIBERESP), IDIVAL, Santander, Spain

^g Division of Rheumatology, Instituto de Investigación Sanitaria-Hospital Clínico Universitario de Santiago, Santiago de Compostela, A Coruña, Spain

^h Department of Rheumatology, Hospital General Universitario Gregorio-Marañón, Madrid, Spain

ⁱ Division of Rheumatology, Complejo Asistencial Universitario de León, León, Spain

^j Department of Rheumatology, Hospital Universitario de la Princesa, Madrid, Spain

^k Rheumatology Division, Hospital Universitario Araba, Vitoria/Gasteiz, Alava, Spain

^l Rheumatology Department, Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain

^m Rheumatology Department, Hospital Universitario La Paz, Madrid, Spain

ⁿ Department of Autoimmune Diseases, Hospital Clínico de Barcelona, Universidad de Barcelona, Barcelona, Spain

^o Rheumatology Department, Hospital Universitario de Bellvitge, Barcelona, Spain

^p Department of Systemic Autoimmune Diseases, Hospital Universitario Valle de Hebron, Barcelona, Spain

^q SERGAS (Servizo Galego de Saude) and IDIS (Instituto de Investigación Sanitaria de Santiago), NEIRID Lab (Neuroendocrine Interactions in Rheumatology and Inflammatory Diseases), Research Laboratory 9, Santiago University Clinical Hospital, Santiago de Compostela, Spain

^r Division of Rheumatology, University and IRCCS Policlinico S. Matteo Foundation, Pavia, Italy

^s School of Medicine, Universidad de Cantabria, Santander, Spain

^t Interstitial Lung Disease Unit, Royal Brompton Hospital, Imperial College, London, United Kingdom

^u Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa



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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

* Corresponding author.

** Co-corresponding author at: Research group on genetic epidemiology and atherosclerosis in systemic diseases and in metabolic diseases of the musculoskeletal system, IDIVAL, Hospital Universitario Marqués de Valdecilla, avenida Cardenal Herrera Oria s/n, Lab. 201/202, 39011 Santander, Spain.

E-mail addresses: rlopezmejias78@gmail.com (R. López-Mejías), miguelaggay@hotmail.com (M.A. González-Gay).

¹ These authors contributed equally to this work.

² These authors shared senior authorship in this study.

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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.

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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 typ-

ical 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 $\geq 38^\circ\text{C}$ 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).

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
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 $P < 0.001$

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 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%, $P = 1.56\text{E-}09$, 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

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]
	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]
	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]
	41:01	0.9 (3)	1.6 (16)	0.54 [0.10–1.90]
HLA-B*41	41:02	0	0.2 (2)	0.00 [0.00–5.57]
	44:02	3.9 (13)	7.1 (69)	0.53 [0.26–0.98]
HLA-B*44	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 $P < 0.001$

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 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 and 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%

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

ASSD versus healthy controls				
Alleles	<i>P</i> -values	<i>P</i> -values conditioned on <i>HLA-DRB1*03:01</i>	<i>P</i> -values conditioned on <i>HLA-DRB1*07:01</i>	<i>P</i> -values conditioned on <i>HLA-B*08:01</i>
<i>HLA-DRB1*03:01</i>	2.48E-06	–	6.34E-05	0.026
<i>HLA-DRB1*07:01</i>	0.0003	0.009	–	0.004
<i>HLA-B*08:01</i>	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.

<i>HLA-DRB1</i>		ASSD anti-Jo-1 positive (2n = 220)	ASSD anti-Jo-1 negative (2n = 116)	OR [95% CI]
<i>DRB1*01</i>	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	–
<i>DRB1*03</i> <i>DRB1*04</i>	03:01	31.8 (70)^a	15.5 (18)^a	2.54 [1.39–4.81]^a
	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	–
	<i>DRB1*07</i>	07:01	6.8 (15)	13.8 (16)
<i>DRB1*08</i>	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]
<i>DRB1*09</i>	09:01	0	0	–
<i>DRB1*10</i>	10:01	0	0	–
<i>DRB1*11</i>	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]
<i>DRB1*12</i>	12:01	0.5 (1)	0	–
<i>DRB1*13</i>	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]
<i>DRB1*14</i>	14:01	1.4 (3)	1.7 (2)	0.79 [0.09–9.57]
	14:04	1.4 (3)	0	–
<i>DRB1*15</i>	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]
<i>DRB1*16</i>	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

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 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-B*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-B*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 myasthenia 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.

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Disclosure of interest

The authors declare that they have no competing interest.

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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>.

References

- [1] Cavagna L, Nuño L, Alberto Scirè C, et al. Clinical spectrum time course in anti-Jo-1 positive antisynthetase syndrome: results from an international retrospective multicentre study. *Medicine* 2015;94:e1144.
- [2] Chatterjee S, Prayson R, Farver C. Antisynthetase syndrome: not just an inflammatory myopathy. *Cleve Clin J Med* 2013;80:655–66.
- [3] Dugar M, Cox S, Limaye V, et al. Clinical heterogeneity and prognostic features of South Australian patients with anti-synthetase autoantibodies. *Intern Med J* 2011;41:674–9.
- [4] González-Gay MA, Montecucco C, Selva-O'Callaghan A, et al. Timing of onset affects arthritis presentation pattern in antisynthetase syndrome. *Clin Exp Rheumatol* 2018;36:44–9.
- [5] Imbert-Masseau A, Hamidou M, Agard C, et al. Antisynthetase syndrome. *Joint Bone Spine* 2003;70:161–8.
- [6] Hervier B, Devilliers H, Stanciu R, et al. Hierarchical cluster and survival analyses of antisynthetase syndrome: phenotype and outcome are correlated with anti-tRNA synthetase antibody specificity. *Autoimmun Rev* 2012;12:210–7.
- [7] Cavagna L, Nuño L, Alberto Scirè C, et al. Serum Jo-1 autoantibody and isolated arthritis in the antisynthetase syndrome: review of the literature and report of the experience of AENEAS collaborative group. *Clin Rev Allergy Immunol* 2017;52:71–80.
- [8] Mirrakhimov A. Antisynthetase syndrome: a review of etiopathogenesis, diagnosis and management. *Curr Med Chem* 2015;22:1963–75.
- [9] The MHC sequencing consortium. Complete sequence and gene map of a human major histocompatibility complex. *Nature* 1999; 401: 921–3.
- [10] Klein J, Sato A. The HLA system. Second of two parts. *N Engl J Med* 2000;343:782–6.
- [11] Unanue ER, Turk V, Neefjes J. Variations in MHC class II antigen processing and presentation in health and disease. *Annu Rev Immunol* 2016;34:265–97.
- [12] O'Hanlon TP, Carrick DM, Targoff IN, et al. Immunogenetic risk and protective factors for the idiopathic inflammatory myopathies: distinct HLA-A, -B, -Cw, -DRB1, and -DQA1 allelic profiles distinguish European American patients with different myositis autoantibodies. *Medicine* 2006;85:111–27.
- [13] Rothwell S, Chinoy H, Lamb JA, et al. Focused HLA analysis in Caucasians with myositis identifies significant associations with autoantibody subgroups. *Ann Rheum Dis* 2019;78:996–1002.
- [14] Chinoy H, Salway F, Fertig N, et al. In adult onset myositis, the presence of interstitial lung disease and myositis specific/associated antibodies are governed by HLA class II haplotype, rather than by myositis subtype. *Arthritis Res Ther* 2006;8:R13.
- [15] Miller FW, Chen W, O'Hanlon TP, et al. Genome-wide association study identifies HLA 8.1 ancestral haplotype alleles as major genetic risk factors for myositis phenotypes. *Genes Immun* 2015;16:470–80.
- [16] Goldstein R, Duvic M, Targoff IN, et al. HLA-D region genes associated with autoantibody responses to histidyl-transfer RNA synthetase (Jo-1) and other translation-related factors in myositis. *Arthritis Rheum* 1990;33:1240–8.
- [17] Szabó K, Bodoki L, Nagy-Vincze M, et al. Effect of genetic and laboratory findings on clinical course of antisynthetase syndrome in a Hungarian cohort. *Biomed Res Int* 2018;2018 [6416378].
- [18] Arnett FC, Targoff IN, Timori T, et al. Interrelationship of major histocompatibility complex class II alleles and autoantibodies in four ethnic groups with various forms of myositis. *Arthritis Rheum* 1996;39:1507–18.
- [19] Love LA, Leff RL, Fraser DD, et al. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. *Medicine* 1991;70:360–74.
- [20] Cavagna L, Caporali R, Abdi-Ali L, et al. Cyclosporine in anti-Jo-1-positive patients with corticosteroid-refractory interstitial lung disease. *J Rheumatol* 2013;40:484–92.
- [21] Sebastiani M, Triantafyllias K, Manfredi A, et al. Nailfold capillaroscopy characteristics of antisynthetase syndrome and possible clinical associations: results of a multicentre international study. *J Rheumatol* 2019;46:279–84.

- [22] Mariampillai K, Granger B, Amelin D, et al. Development of a new classification system for idiopathic inflammatory myopathies based on clinical manifestations and myositis-specific autoantibodies. *JAMA Neurol* 2018;75:1528–37.
- [23] Bartoloni E, Gonzalez-Gay MA, Scirè C, et al. Clinical follow-up predictors of disease pattern change in anti-Jo-1 positive anti-synthetase syndrome: results from a multicentre, international and retrospective study. *Autoimmun Rev* 2017;16:253–7.
- [24] 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.
- [25] Chinoy H, Adimulam S, Marriage F, et al. Interaction of HLA-DRB1*03 and smoking for the development of anti-Jo-1 antibodies in adult idiopathic inflammatory myopathies: an European-wide case study. *Ann Rheum Dis* 2012;71:961–5.
- [26] Wandstrat A, Wakeland E. The genetics of complex autoimmune diseases: non-MHC susceptibility genes. *Nat Immunol* 2001;2:802–9.
- [27] Criswell LA, Pfeiffer KA, Lum RF, et al. Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. *Am J Hum Genet* 2005;76:561–71.
- [28] Zhernakova A, van Diemen CC, Wijmenga C. Detecting shared pathogenesis from the shared genetics of immune-related diseases. *Nat Rev Genet* 2009;10:43–55.
- [29] Davidson A, Diamond B. Autoimmune diseases. *N Engl J Med* 2001;345:340–50.
- [30] Newton JL, Harney SMJ, Wordsworth BP, et al. A review of the MHC genetics of rheumatoid arthritis. *Genes Immun* 2004;5:151–7.
- [31] Price P, Witt C, Allcock R, et al. The genetic basis for the association of the 8.1 ancestral haplotype (A1, B8, DR3) with multiple immunopathological diseases. *Immunol Rev* 1999;167:257–74.
- [32] Candore G, Lio D, Colonna Romano G, et al. Pathogenesis of autoimmune diseases associated with 8.1 ancestral haplotype: effect of multiple gene interactions. *Autoimmun Rev* 2002;1:29–35.
- [33] Christiansen FT, Zhang WJ, Griffiths M, et al. Major histocompatibility complex (MHC) complement deficiency, ancestral haplotypes and systemic lupus erythematosus (SLE): C4 deficiency explains some but not all of the influence of the MHC. *J Rheumatol* 1991;18:1350–8.
- [34] Tan FK, Arnett FC. The genetics of lupus. *Curr Opin Rheumatol* 1998;10:399–408.
- [35] Kallenberg CG, Van der Voort-Beelen JM, D'Amaro J, et al. Increased frequency of B8/DR3 in scleroderma and association of the haplotype with impaired cellular immune response. *Clin Exp Immunol* 1981;43:478–85.
- [36] Vandiedonck C, Beaurain G, Giraud M, et al. Pleiotropic effects of the 8.1 HLA haplotype in patients with autoimmune myasthenia gravis and thymus hyperplasia. *Proc Natl Acad Sci U S A* 2004;101:15464–9.
- [37] Degli-Esposti MA, Andreas A, Christiansen FT, et al. An approach to the localisation of the susceptibility genes for generalised myasthenia gravis by mapping recombinant ancestral haplotypes. *Immunogenetics* 1992;35:355–64.
- [38] Juge P-A, Borie R, Kannengiesser C, et al. Shared genetic predisposition in rheumatoid arthritis-interstitial lung disease and familial pulmonary fibrosis. *Eur Respir J* 2017;49 [1602314].
- [39] Juge P-A, Lee JS, Ebstain E, et al. *MUC5B* promoter variant and rheumatoid arthritis with interstitial lung disease. *N Engl J Med* 2018;379:2209–19.
- [40] López-Mejías R, Remuzgo-Martínez S, Genre F, et al. Influence of *MUC5B* gene on antisynthetase syndrome. *Sci Rep* 2020;10:1415.