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Multiple Autoimmune Syndrome

Clinical, immunological and genotypic characterization of a group of patients from Unidade de Imunologia Clínica – Centro Hospitalar do Porto

MARIANA BRANDÃO FIDALGO

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Eles não sabem que o sonho
é uma constante da vida
tão concreta e definida
como outra coisa qualquer.

(...)

Eles não sabem, nem sonham,
que o sonho comanda a vida,
que sempre que um homem sonha
o mundo pula e avança
como bola colorida
entre as mãos de uma criança.

António Gedeão

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E por tudo o que há entre nós

Ao Zé, meu amor-perfeito. Porque me centra quando tudo é tempestade. Porque fecha o mundo
num abraço. Pela liberdade para perseguir o impossível –
sem ele tudo seria apenas sonho.
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Pela vontade que me traz de ser mais e melhor.
E para lhe mostrar que todos os sonhos são nossos e possíveis.

Multiple Autoimmune Syndrome: clinical, immunological and genotypic characterization of a group of patients from Unidade de Imunologia Clínica – Centro Hospitalar do Porto

Mariana Brandão Fidalgo[1]
Lúcia Raquel Moreira Faria[2,3]
Cláudia Alexandra Moreira de Carvalho[3,4]
Graziela Maria Sousa Carvalheiras[2]
Denisa Maria de Melo Vasques Mendonça[5,6]
Berta Martins da Silva[3,4]
Carlos Alberto da Silva e Vasconcelos[2,3]

1. Mestrado Integrado em Medicina – Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto
2. Unidade de Imunologia Clínica – Centro Hospitalar do Porto
3. Unit for Multidisciplinary Research in Biomedicine
4. Laboratório de Imunogenética – Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto
5. Departamento de Estudos de Populações – Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto
6. EpiUnit, Instituto de Saúde Pública – Universidade do Porto

Instituto de Ciências Biomédicas Abel Salazar - Universidade do Porto
Rua de Jorge Viterbo Ferreira n.º 228, 4050-313 Porto, Portugal.

Centro Hospitalar do Porto
Largo Prof. Abel Salazar, 4099-001 Porto, Portugal.

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Abstract

Introduction: The existence of subphenotypes common to several autoimmune diseases (AIDs) suggests a shared physiopathology - autoimmune tautology. Multiple Autoimmune Syndrome (MAS) - the coexistence of three or more AIDs in one person-, best illustrates that polyautoimmunity is more than a coincidence.

Objectives: Characterize and compare the monoautoimmune and MAS patients. Understand if clustering of AIDs leads to differences in disease severity, autoantibodies expression or genetic polymorphisms that could be markers for polyautoimmunity.

Methods: Currently adult patients were selected from unit cohort. MAS was assumed when ≥3 AIDs were present. 331 patients were included after exclusion criteria: having two AIDs or undetermined diagnosis. Clinical and immunological data were collected from medical files. HLA-DRB1 was genotyped by PCR-SSP methodology and PTPN22(rs2476601) polymorphisms by TaqMan Real Time PCR. Data were analysed using Chi-Square, Fisher's exact tests and logistic regression. Odds ratios (OR) and 95% confidence intervals were calculated.

Results: In comparison with control population:

Elevated frequencies: HLA-DRB1*03 in study cohort (OR=3.68,p<0.001) and in monoautoimmune SLE (OR=2.79,p<0.001) and SjS (OR=8.27,p<0.001); HLA-DRB1*15 in monoautoimmune SjS (OR=2.39,p=0.011); HLA-DRB1*16 in MAS SLE (OR=2.67,p=0.031); PTPN22_T in all groups except monoautoimmune SjS and triple positive systemic MAS.

Diminished frequencies: HLA-DRB1*11 in study cohort (OR=0.57,p=0.013), in MAS SLE (OR=0.39,p=0.031) and monoautoimmune SjS (OR=0.10,p=0.005); HLA-DRB1*13 in study cohort (OR=0.52,p=0.001) and in monoautoimmune SLE (OR=0.53,p=0.009) and SjS (OR=0.38,p=0.031); HLA-DRB1*14 in study cohort (OR=0.32,p=0.013) and monoautoimmune SLE (OR=0.21,p=0.021);

SLE group: HLA-DRB1*07 frequency was higher in monoautoimmune patients (OR=0.43,p=0.023). MAS patients had significantly more NPSLE (OR=2.99,p<0.001), subacute cutaneous lesions (OR=2.30,p=0.037), muscle&tendon (OR=2.00,p=0.045), and haematological (OR=3.18,p=0.006) involvement and Raynaud's (OR=2.94,p<0.001).

SjS group: MAS patients had more frequently cryoglobulins (OR=2.96,p=0.030), low complement (OR=2.43,p=0.030) and Raynaud's (OR=4.38,p<0.001); monoautoimmune patients had more parotid enlargement (OR=0.12,p<0.001).

APS group: MAS patients had more non-thrombotic manifestations (OR=4.69,p=0.020) and Raynaud's (OR=9.12,p<0.001).

Triple positive systemic MAS (SLE+SjS+APS) had more frequently severe kidney involvement (OR=11.67,p=0.021) and CNS thrombosis (OR=4.44,p=0.009). Anti-U1RNP increased frequency was transversally attributable to MAS.

Conclusions: The coexistence of AIDs contributes to a more severe disease course. We confirmed previously established genetic risk and protection factors and suggest a new protective one - HLA-DRB1*14. HLA-DRB1*07 and anti-U1RNP could be markers for mono and polyautoimmunity, respectively; HLA-DRB1*13 could be a predictor for vascular risk in patients with multiple AIDs. PTPN22(rs2476601) polymorphism could be associated with less severe disease.

Key words: multiple autoimmune syndrome, polyautoimmunity, systemic lupus erythematosus, Sjögren's syndrome, antiphospholipid syndrome; HLA-DRB1, PTPN22, triple positive systemic MAS.

Introduction

Autoimmune diseases (AIDs) are complex, chronic diseases due to the loss of immunological tolerance to self-antigens.[1-12] AIDs are a heterogeneous group, comprised by more than 80 diseases[13] with different phenotypes (organ-specific vs. systemic or non-organ specific)[14] and an estimated world prevalence of 3-9.4%.[14-17] This represents a significant burden on social and medical resources, with direct and indirect costs and impact on quality of life.[3-5,18] The ability to predict these diseases in a pre-symptomatic stage or to predict their evolution would represent an important step towards primary, secondary and tertiary prevention.

Monoautoimmunity and polyautoimmunity

The vast majority of AIDs occur as one single disease in one person (monoautoimmunity), presenting with characteristic clinical and immunological markers that are disease-specific. However, the existence of clinical subphenotypes common to several AIDs suggests that they might share physiopathological mechanisms (genetic and environmental triggering factors) – meaning they would have a common origin: autoimmune tautology.[4-5,8-9,11-12,19-23] This is corroborated by three levels of evidence: 1) clinical observations indicating a possible shift from one disease to another over time or the coexistence of more than one AID in a single patient (polyautoimmunity) or family (familial autoimmunity); 2) known shared pathophysiological mechanisms between AIDs and 3) evidence implying common genetic factors.[8-9,19,21]

The estimated world prevalence of polyautoimmunity is 0.5%, which means that approximately 4.4% of autoimmune patients presents more than one AID.[16] Multiple Autoimmune Syndrome (MAS), which represents the coexistence of three or more AIDs in one person,[4-5,7,9] best illustrates that polyautoimmunity is more than a coincidence.[5,9,22,24-26]

"Chaperones" of autoimmunity[3,5,19-20,27] are diseases that, when present, signal an increased probability of other AIDs[27-28], given that they are the main aggregators of polyautoimmunity (i.e., the more frequent AIDs in clusters of autoimmunity). Those AIDs are autoimmune thyroid disease (AITD), systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS) and antiphospholipid syndrome (APS).

Monogenic vs. polygenic diseases

The vast majority of AIDs is polygenic – therefore, it is not possible to attribute direct genetic causality. The genes involved in autoimmunity have a pleiotropic behaviour[2,6] – according to Becker's "common variants/multiple diseases" hypothesis, "complex phenotypes are not unique entities but are mosaics of common disease specific alleles

and non-disease specific modifying alleles in the population, influenced by a vast array of environmental factors."[2,5,29-30] The genetic basis of autoimmunity is even more complex, given its epistasis – i.e., the effect of a gene is determined by its interaction with one or more different genes. Therefore, autoimmunity arises from the genetic pleiotropism and epistasis, environmentally modified. As such, the identification of culprit genetic polymorphisms and their possible interactions is a fundamental step to understanding the autoimmune phenomenon.

Genetics and polyautoimmunity

The pathologic mechanism responsible for the coexistence of AIDs is yet to be understood. The fact that several autoimmune phenotypes share susceptibility genes suggests a common genetic background. The phenomenon of familial autoimmunity and the juxtaposition of chromosomic regions associated with AIDs (for example, the 6p21.3 region) support that hypothesis. However, it is important to consider that genetic susceptibility to AIDs might derive not only from the presence of risk alleles, but also from the lack of protective ones.[14]

The most important genetic risk factor is the Major Histocompatibility Complex (MHC)[14] and its Human Leukocyte Antigen (HLA) region is one of the most extensively investigated regions in the human genome.[10] Studies and genetic mapping have reported an association between several HLA alleles on classes I and II and AIDs.[14] Two etiopathogenic models provide possible explanations for the increased AID risk associated with specific HLA alleles, the molecular mimicry hypothesis and the central selection failure hypothesis.[12] In the latter, it has been proposed that specific peptide-HLA class II combinations affect T-cell development and/or tolerance, which may confer susceptibility to AIDs.[31] A recent study suggests a third possibility, by stating that misfolded proteins complexed with certain HLA class II alleles might affect susceptibility to AIDs by acting as specific targets for autoantibodies.[31-32] Despite the fact that the exact mechanisms by which HLA class II polymorphisms influence susceptibility to AIDs is still unknown,[10] the association of specific haplotypes (such as A1-B8-DR3-DQ2) with different AIDs is well documented[2,6,12,17,31-37] and thus reinforces HLA as a major genetic contributor to AID susceptibility.[36]

The emergence of genome-wide association studies (GWAS) led to the identification of other susceptibility genes for AIDs[17,20,35,38], namely several single nucleotide polymorphisms (SNPs).[14-15,17,35,39-42] Of these, the most important and best studied is probably PTPN22,[17,43] which encodes the phosphatase Lyp (lymphoid-specific tyrosine phosphatase), a protein with an important suppressive role on the immune system.[43] Carriers of the Lyp620W (or rs2476601) polymorphism show attenuated T and B cell

receptor signalling and an abnormal immune response.[44] This polymorphism has been associated with increased susceptibility to several AIDs[15,39,42,44-48] and possibly to MAS as well.[1]

Given their proven role in susceptibility to AIDs, the HLA and PTPN22 genes could, arguably, be used as autoimmunity markers.

The purpose of the present study was to characterize and compare the two extremes of autoimmunity, the monoautoimmune patient and the MAS patient, and to provide a global perspective (clinical, immunological and genetic) of these subgroups. We wanted to understand if the clustering of AIDs led to a less severe phenotype, giving the possible scattering of the immune system, or if, on the contrary, it resulted in a more serious disease course and organ involvement. Parallel to that, we aimed to find if the co-occurrence of the three chaperones of systemic autoimmunity considered in this study (SLE, SjS and APS) translated in different disease severity, when compared with other MAS patients. For convenience purposes, we propose the nomenclature “triple positive systemic MAS” for the group of patients with SLE and SjS and APS (with or without additional AIDs).

Our final goal was to find any significant differences in autoantibodies expression or genetic polymorphisms that could be used as markers for polyautoimmunity and allow preventive interventions.

Methods

Patients were selected from the Unidade de Imunologia Clínica (UIC) – Centro Hospitalar do Porto’s systemic lupus erythematosus (SLE), Sjögren’s syndrome (SjS) and antiphospholipid syndrome (APS) cohorts. A total of 1050 individual medical records were reviewed, resulting in a selection of 450 potential study patients. Inclusion criteria: a diagnosis of at least one of the systemic aggregation AIDs (SLE or SjS or APS). Exclusion criteria: underage patients; diagnosis of two AIDs. Diagnoses were established based on consensual criteria: the 1997 ACR classification criteria for SLE[49], the 2012 ACR classification criteria for SjS[50] and the 2012 Sydney classification criteria for APS[51].

Patients were recruited by telephone and blood collection synchronized with routine follow-up schedules. Genotyping was done in the Laboratório de Imunogenética of the Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto. The HLA-DRB1 and PTPN22 frequencies obtained were compared with a control population, consisting of 282 unrelated healthy individuals from the same geographic area (north of Portugal).

Clinical and immunological data

Patient paper and electronic medical records were consulted for collection of clinical data over the follow-up years. For SLE, we constructed a short version of the Cumulative SLE Manifestations for Genetic Studies checklist to guide the data collecting process (see Appendix 1). For SjS, data was collected about the presence of sicca syndrome and bad prognosis criteria (parotid enlargement, adenopathies, cutaneous vasculitis, cryoglobulinemia, hypocomplementemia, hypergammaglobulinemia, pulmonary or nervous system involvement and past or present lymphoma). For APS, data was collected about the occurrence of thrombotic and non-thrombotic events, according to the Sydney classification criteria[51] and also of other events not included in said criteria but referred by Graham Hughes as related to APS[52] – namely patient-reported memory problems, visual disturbances, balance impairment or vertigo episodes, sleep disturbances, avascular hip necrosis, frequent fractures, seizures and psychiatric problems. Immunologic data comprised all antibodies associated with the four chaperones of autoimmunity mentioned above.

HLA-DRB1 Genotyping

Peripheral blood samples were collected in EDTA. Genomic DNA was obtained from proteinase-K-treated peripheral blood leukocytes by using a Salting-Out procedure.[53] Low-resolution genotyping for HLA-DRB1 locus was performed using polymerase chain reaction and sequence-specific primers (PCR-SSP), based on methods previously described.[54] PCR products were visualized under ultraviolet light after running a 1.5% agarose gel containing ethidium bromide.[12]

PTPN22 Genotyping

Genomic DNA was extracted from peripheral white blood cells following standard techniques for salting-out procedure. The SNP were genotyped using pre-designed TaqMan® allelic discrimination assays from Applied Biosystems (Foster City, CA, USA) in a Rotor Gene 6000 Real-Time PCR machine (Corbett Life Science). Genotyping of the PTPN22 (rs2476601) genetic variant, located within the 1p13 (PTPN22 gene) region, was carried out.

Statistical Analysis

Significant differences in the percentages between groups were analysed using Pearson Chi-Square and Fisher's exact tests. Odds ratios (ORs) and the 95% confidence intervals (95% CI) were calculated; a p value less than 0.05 was considered to indicate

statistical significance. To analyse for confounding factors, logistic regression was used – we considered as confounding factors different AIDs that could justify the differences found between subgroups. Data were analysed with IBM SPSS23® software.

Results

The patient recruitment process resulted in a study population of 331 patients, all of European ascendancy, whose distribution is shown in Figure 1. Due to technical issues, we were not able to genetically characterize every sample; the total number of patients typed for HLA-DRB1 was 299 and PTPN22 280.

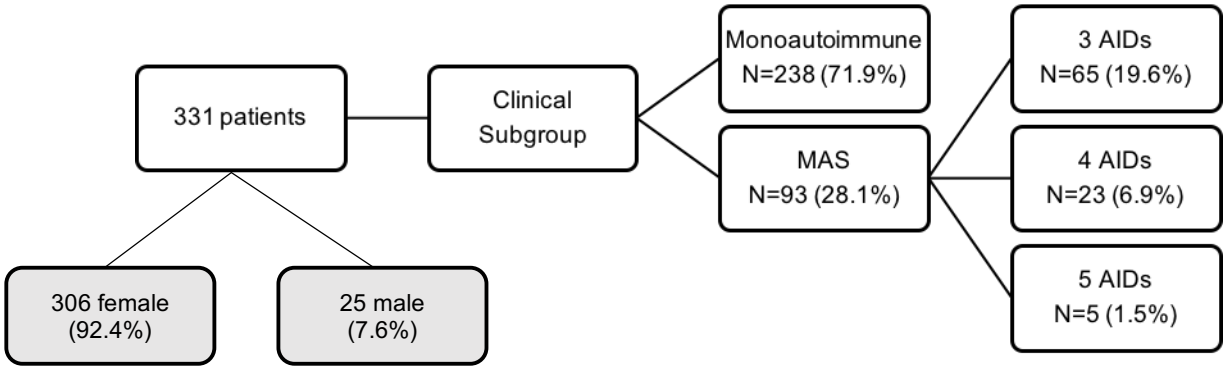

Figure 1: Characterization of study cohort
(MAS=multiple autoimmune syndrome; AID=autoimmune disease)

The mean age at first AID diagnosis was 33.79y (SD 14.03; range 7-79y), with a mean global follow-up time of 15.25y (SD 8.19) and a range of [2-46] follow-up years.

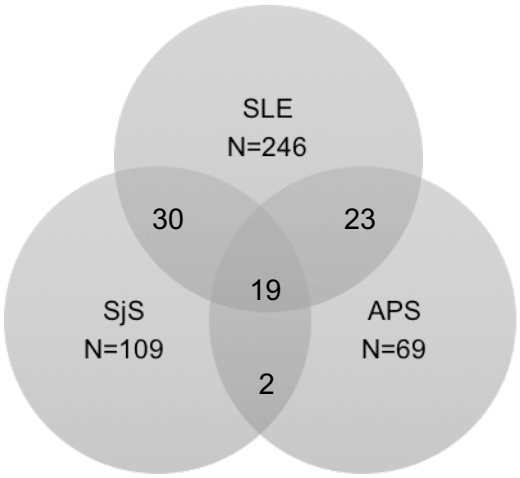

Figure 2: Number of patients per chaperone AID

The number of patients presenting with each of the systemic chaperones of AID can be seen in Figure 2. Twenty-six other AIDs were present (see Appendix 2, Table XVI), the most frequent being AITD (23 patients), Systemic sclerosis/CREST, Psoriasis and Primary biliary cirrhosis (15 patients each) and Rheumatoid arthritis and Autoimmune hepatitis (8 patients each).

The HLA class II allelic frequencies in the study cohort were different from the control population, as shown in Table I:

Table I: HLA-DRB1 allelic frequencies in control population and study cohort

Class II HLA	Control population	Study cohort		
	%	%	Odds ratio	p
DRB1*01	23.40	**16.72**	**0.66**	**0.044**
DRB1*03	15.60	**40.47**	**3.68**	**<0.001**
DRB1*04	24.47	18.39	-	n.s.
DRB1*07	25.53	25.08	-	n.s.
DRB1*08	8.51	9.36	-	n.s.
DRB1*09	4.96	**0.33**	**0.06**	**<0.001**
DRB1*10	3.90	3.34	-	n.s.
DRB1*11	19.50	**12.04**	**0.57**	**0.013**
DRB1*12	3.19	2.21	-	n.s.
DRB1*13	29.79	**18.06**	**0.52**	**0.001**
DRB1*14	6.03	**2.01**	**0.32**	**0.013**
DRB1*15	19.90	25.08	-	n.s.
DRB1*16	4.61	7.69	-	n.s.

The PTPN22 allelic frequencies were also significantly discrepant between the study cohort and the control population, as Table II shows (the T allele corresponds to the rs2476601 polymorphism mentioned above).

Table II: PTPN22 allelic frequencies in control population and study cohort

PTPN22 alleles	Control population	Study cohort (N=280)		
	%	%	OR	p
C	93.1	85.2	0.43	<0.001
T	6.9	14.8	2.34	

There were some significant associations between HLA class II alleles and antibody expression - Table III. All variables not featured had no statistically significant differences.

Table III: Associations between genetic variables and antibody expression

Genetic variables		**Immunologic variables**		
Class II HLA		**Antibodies**	OR [95%CI]	p
Susceptibility	DRB1*03	anti-Ro/SSA	1.78 [1.11-2.84]	0.017
		anti-La/SSB	3.58 [2.03-6.34]	<0.001
	DRB1*04	aβ2GPI IgG	2.54 [1.26-5.13]	0.008
		aβ2GPI IgM	2.06 [1.11-3.80]	0.020
	DRB1*07	anti-U1RNP	1.95 [1.12-3.39]	0.017
	DRB1*08	anti-dsDNA	2.83 [1.11-7.20]	0.024
		low C4	2.34 [1.00-5.50]	0.046
		anti-Sm	3.80 [1.68-8.59]	0.001
		anti-ribosomal P	4.69 [1.24-17.73]	0.034
	DRB1*10	aCL IgM	5.00 [1.37-18.25]	0.016
		aβ2GPI IgM	4.22 [1.16-15.37]	0.028
	DRB1*13	aβ2GPI IgM	1.88 [1.00-3.52]	0.048
		anti-phosphatidylserine IgG	9.46 [2.04-43.84]	0.006
	DRB1*16	anti-Ro/SSA	3.53 [1.27-9.83]	0.011
Protection	DRB1*03	low C3	0.60 [0.37-0.96]	0.033
		aCL IgG	0.44 [0.24-0.83]	0.009
		aCL IgM	0.53 [0.30-0.94]	0.028
	DRB1*04	anti-Ro/SSA	0.48 [0.26-0.87]	0.015
		anti-La/SSB	0.36 [0.15-0.88]	0.021
		rheumatoid factor	0.42 [0.21-0.83]	0.012
	DRB1*11	ANA	0.17 [0.04-0.80]	0.040

	DRB1*15	low C3	0.51 [0.30-0.87]	0.013
		aβ2GPI IgG	0.24 [0.08-0.70]	0.005
	DRB1*16	aβ2GPI IgG	0.83 [0.79-0.88]	0.032
PTPN22				
Susceptibility	PTPN22_T	anti-dsDNA	2.07 [1.05-4.07]	0.032
Protection	PTPN22_T	anti-La/SSB	0.31 [0.11-0.89]	0.022

(aCL=anticardiolipin; aβ2GPI=anti-β2glycoprotein I; ANA=antinuclear antibodies)

Systemic Lupus Erythematosus

The SLE group's distribution is represented in Figure 3.

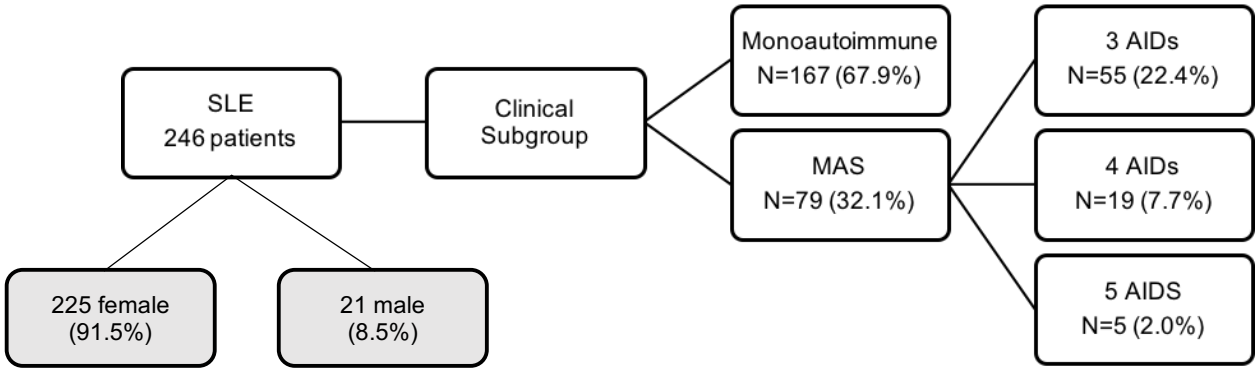

Figure 3: Characterization of SLE group in the study cohort
(MAS=multiple autoimmune syndrome; AID=autoimmune disease)

The mean age at onset of SLE symptoms was 28.21y (SD 12.21, span 6-62) and at SLE diagnosis 31.61y (SD 12.72; span 7-65yo); mean follow-up time was 15.32y (SD 8.00, span 1-39y).

The HLA class II allelic frequencies for the SLE group and its mono and MAS subgroups, are shown in Table IV; Table V displays the allelic frequencies for PTPN22 (rs2476601) polymorphism. Both tables establish comparison with the allelic frequencies in the control population.

Table IV: HLA class II allelic frequencies in control population and SLE group

Class II HLA	Control population	SLE group								
		All			Mono			MAS		
	%	%	OR	p	%	OR	p	%	OR	p
DRB1*01	23.40	17.04	-	n.s.	16.99	-	n.s.	17.14	-	n.s.
DRB1*03	15.60	**36.77**	**3.15**	**<0.001**	**34.00**	**2.79**	**<0.001**	**42.86**	**4.06**	**<0.001**
DRB1*04	24.47	17.94	-	n.s.	16.99	-	n.s.	20.00	-	n.s.
DRB1*07	25.53	25.56	-	n.s.	30.07	-	n.s.	15.71	-	n.s.
DRB1*08	8.51	9.87	-	n.s.	8.50	-	n.s.	12.86	-	n.s.
DRB1*09	4.96	**0.45**	**0.09**	**0.003**	**0.70**	**0.13**	**0.019**	0.00	-	n.s.
DRB1*10	3.90	3.14	-	n.s.	3.27	-	n.s.	2.86	-	n.s.
DRB1*11	19.50	13.45	-	n.s.	15.69	-	n.s.	**8.57**	**0.39**	**0.031**
DRB1*12	3.19	2.24	-	n.s.	2.61	-	n.s.	1.43	-	n.s.
DRB1*13	29.79	**19.28**	**0.56**	**0.007**	**18.30**	**0.53**	**0.009**	21.43	-	n.s.
DRB1*14	6.03	**1.79**	**0.29**	**0.018**	**1.31**	**0.21**	**0.021**	2.86	-	n.s.
DRB1*15	19.90	24.22	-	n.s.	24.18	-	n.s.	24.29	-	n.s.
DRB1*16	4.61	8.97	-	n.s.	7.84	-	n.s.	**11.43**	**2.67**	**0.031**

Table V: PTPN22 allelic frequencies in control population and SLE group

PTPN22 alleles	Control population	SLE group								
		All			Mono			MAS		
	%	%	OR	p	%	OR	p	%	OR	p
C	93.1	83.6	0.38	<0.001	84.1	0.39	<0.001	82.4	0.35	<0.001
T	6.9	16.4	2.65		15.9	2.55		17.6	2.88	

In the SLE group, the comparison between monoautoimmune and MAS patients yielded some differences, which are presented in Table VI. All variables not featured had no statistically significant differences.

Analysing potential confounding factors, the higher incidence of the following clinical and immunological variables can be attributed to MAS (and not to an individual AID that might coexist): hematologic involvement, Raynaud's phenomenon and anti-CCP. All aPLs are due to APS co-occurrence; anti-peroxidase is due to AITD. Anti-Ro/SSA, anti-La/SSB and anti-U1RNP are mostly due to coexisting SjS, but MAS retains influence.

Given the found differences, we aimed to understand if some immunologic and/or genetic variable could account for them - Table VI also presents these possible risk (positively associated) and protection (negatively associated) factors.

The statistical values for these and other associations can be found in Appendix 3, Tables XVII-XXI.

Table VI: SLE group significant differences between mono and MAS subgroups, with corresponding confounding factors and risk/protection genetic and immunologic factors

Clinical, immunologic and genetic variables		SLE Mono vs. MAS			Confounding factors		MAS SLE				Mono SLE			
		favouring	OR [95%CI]	p	Which	favouring	Genetic		Immunologic		Genetic		Immunologic	
Organ involvement	NPSLE						Risk	Protection	Risk	Protection	Risk	Protection	Risk	Protection
	- global	MAS	2.99 [1.60-5.60]	<0.001	-		-	-	-	-	-	-	aPL, aCL IgM	anti-C1q, anti-Sm
	- central focal	MAS	2.26 [1.14-4.48]	0.018	-		-	DRB1*15	-	-	-	-	aPL, aCL IgM	anti-U1RNP
	- central diffuse	MAS	2.83 [1.01-7.90]	0.040	-		-	-	-	-	-	-	-	-
	Mucocutaneous - subacute	MAS	2.30 [1.04-5.10]	0.037	-		DRB1*16	-	anti-Ro/SSA	-	-	-	anti-C1q	-
	Musculoskeletal - muscle/tendon	MAS	2.00 [1.01-5.97]	0.045	-		-	-	anti-La/SSB	-	-	-	-	aPL
	Haematological													
	- global	MAS	3.18 [1.35-7.49	0.006	SjS	MAS	-	-	aCL IgG	-	-	-	a-dsDNA, low C3&4, a-nucleos.	
	- thrombocytopenia	MAS	1.89 [1.08-3.31]	0.025			-	-	anti-CCP	-	-	-		
	Other - Raynaud's phenomenon	MAS	2.94 [1.68-5.14]	<0.001	SjS	MAS>SjS	-	-	anti-Sm, aβ2GP I IgG	-	-	DRB1*08	anti-SSA, a-U1RNP, a-nucleos.	aPL
Antibodies	anti-Ro/SSA	MAS	3.08 [1.75-5.41]	<0.001	SjS	SjS>MAS	DRB1*16	-			-	-		
	anti-La/SSB	MAS	2.83 [1.42-5.66]	0.002	SjS	SjS>MAS	DRB1*03	-			DRB1*03	-		
	anti-U1RNP	MAS	1.80 [1.03-3.14]	0.039	SjS	SjS>MAS	DRB1*07	-			-	-		
	anti-CCP	MAS	R(MAS) = 3.15 [2.19-4.52]	0.039	RA	MAS	-	-			-	-		
	anti-peroxidase	MAS	4.02 [1.31-12.38]	0.010	AITD	AITD	-	DRB1*13			-	-		
	aPL													
	- global	MAS	4.15 [2.29-7.51]	<0.001	APS	APS	-	-			-	-		
	- aCL IgG	MAS	3.15 [1.71-5.82]	<0.001	APS	APS	-	DRB1*03			-	-		
	- aβ2GPI IgG	MAS	3.62 [1.77-7.38]	<0.001	APS	APS	DRB1*04	-			DRB1*12	-		
	- aβ2GPI IgM	MAS	1.99 [1.12-3.56]	0.019	APS	APS	DRB1*13	-			-	-		
HLA	DRB1*07	mono	0.43 [0.21-0.90]	0.023										

The analysis of confounding factors was accomplished through logistic regression; the table presents the confounding factors that had statistical significance (under "Which") and the variable with highest influence (under "favouring"). (NPSLE=neuropsychiatric SLE; RA=rheumatoid arthritis; aPL=antiphospholipid antibodies; aCL=anticardiolipin antibodies; aβ2GPI= anti-β2 Glycoprotein I antibodies; a-nucleos.=anti-nucleosome antibodies)

Sjögren's syndrome

The SjS subgroup's distribution is represented in Figure 4.

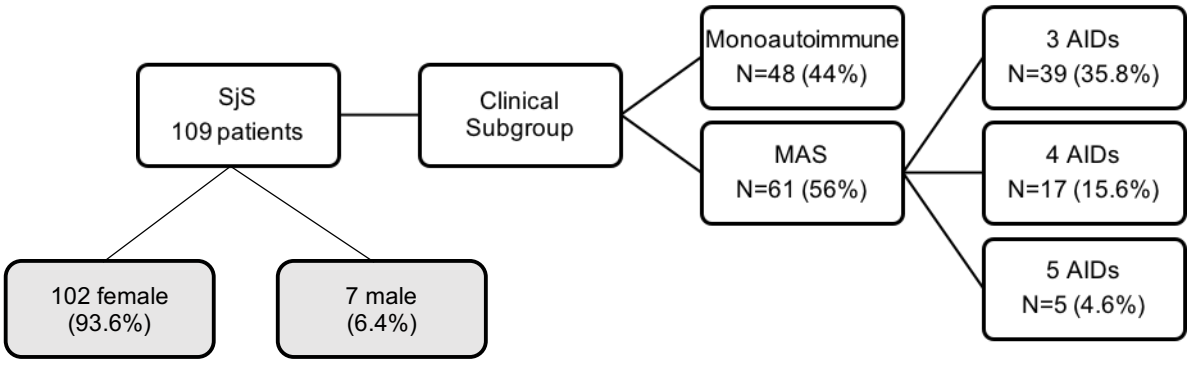

Figure 4: Characterization of SjS group in the study cohort
(MAS=multiple autoimmune syndrome; AID=autoimmune disease)

The mean age at onset of SjS symptoms was 42.76 (SD 13.88, span 5-75yo) and at SjS diagnosis was 45.08y (SD 13.58; span 7-79yo); mean follow-up time was 7.94y (SD 6.27, span 0-35y).

Table VII presents the results for the HLA class II allelic frequencies in the SjS group and its mono and MAS subgroups. In Table VIII the allelic frequencies for PTPN22 (rs2476601) polymorphism, compared to those of the control population.

Table VII: HLA class II allelic frequencies in control population and Sjögren's syndrome study group

Class II HLA	Control population	SjS group								
		All			Mono			MAS		
	%	%	OR	p	%	OR	p	%	OR	p
DRB1*01	23.40	16.00	-	n.s.	16.28	-	n.s.	15.79	-	n.s.
DRB1*03	15.60	**53.00**	**6.10**	**<0.001**	**60.50**	**8.27**	**<0.001**	**47.37**	**4.87**	**<0.001**
DRB1*04	24.47	16.00	-	n.s.	11.63	-	n.s.	19.30	-	n.s.
DRB1*07	25.53	18.00	-	n.s.	18.60	-	n.s.	17.54	-	n.s.
DRB1*08	8.51	11.00	-	n.s.	9.30	-	n.s.	12.28	-	n.s.
DRB1*09	4.96	0.00	0.023	n.s.	0.00	-	n.s.	0.00	-	n.s.
DRB1*10	3.90	4.00	-	n.s.	4.65	-	n.s.	3.51	-	n.s.
DRB1*11	19.50	**6.00**	**0.26**	**0.002**	**2.33**	**0.10**	**0.005**	8.77	-	n.s.
DRB1*12	3.19	1.00	-	n.s.	2.33	-	n.s.	0.00	-	n.s.
DRB1*13	29.79	**18.00**	**0.52**	**0.022**	**13.95**	**0.38**	**0.031**	21.05	-	n.s.
DRB1*14	6.03	**1.00**	**0.16**	**0.041**	0.00	-	n.s.	1.75	-	n.s.
DRB1*15	19.90	**31.00**	**1.81**	**0.0225**	**37.21%**	**2.39**	**0.011**	26.32	-	n.s.
DRB1*16	4.61	7.00	-	n.s.	4.65%	-	n.s.	8.77	-	n.s.

Table VIII: PTPN22 allelic frequencies in control population and Sjögren's syndrome study group

PTPN22 alleles	Control population	SjS group								
		All			Mono			MAS		
	%	%	OR	p	%	OR	p	%	OR	p
C	93.1	90.3	0.69	0.023	91.8	-	n.s.	88.9	0.59	0.001
T	6.9	9.7	1.45		8.2	-	n.s.	11.1	1.69	

In the SjS group, the comparison between monoautoimmune and MAS patients yielded some differences – Table IX. There were no significant differences in genetic polymorphisms.

Analysing potential confounding factors, none of the differences can be solely attributed to MAS. Antiphospholipid antibodies are attributable to coexistent APS, although MAS retains influence; anti-U1RNP is mostly due to SLE, but also to MAS. Raynaud's phenomenon is mostly due to SLE, but also to MAS and systemic sclerosis. Anti-dsDNA, low complement and anti-nucleosome are all due to coexisting SLE.

Given the differences found, we aimed to understand if some immunologic and/or genetic variable could account for them - Table IX also presents these possible risk (positively associated) and protection (negatively associated) factors.

The statistical values for these and other associations can be found in Appendix 4, Tables XXII-XXVI.

Table IX: SjS group significant differences between mono and MAS subgroups, corresponding confounding factors and risk/protection genetic and immunologic factors

Clinical, immunologic and genetic variables		SjS Mono vs. MAS			Confounding factors		MAS SjS				Mono SjS			
		favouring	OR [95%CI]	p	Which	favouring	Genetic		Immunologic		Genetic		Immunologic	
	Bad prognosis						Risk	Protection	Risk	Protection	Risk	Protection	Risk	Protection
	- parotid enlargement	mono	0.12 [0.03-0.45]	<0.001	-	-	-	-	anti-thyroglobulin	-	-	-	-	-
	- cryoglobulins	MAS	2.96 [1.09-8.01]	0.030	-	-	-	-	anti-centromere	-	-	-	-	-
	- low complement	MAS	2.43 [1.08-5.45]	0.030	SLE	SLE	-	-	anti-dsDNA, aβ2GPI IgG	-	-	-	aPL	-
	Other - Raynaud's phenomenon	MAS	4.38 [1.93-9.94]	<0001	SLE	SLE	-	-	anti-U1RNP	-	-	-	-	-
Antibodies	anti-dsDNA	MAS	11.58 [4.03-33.28]	<0.001	SLE	SLE	-	-			-	-		
	low C3	MAS	4.04 [1.77-9.24]	0.002	SLE	SLE	PTPN22_T	-			-	DRB1*03		
	anti-U1RNP	MAS	3.68 [1.52-8.89]	0.039	SLE	SLE	DRB1*07 DRB1*15	-			-	-		
	anti-nucleosome	MAS	1.95 [1.42-2.67]	0.039	SLE	SLE	DRB1*01	-			-	-		
	rheumatoid factor	mono	0.42 [0.19-0.96]	0.010	-	-	-	DRB1*04			-	-		
	aPL													
	- global	MAS	6.39 [2.56-15.98]	<0.001	APS	APS	DRB1*13	DRB1*08			-	DRB1*15		
	- aCL IgG	MAS	14.67 [1.86-15.77]	0.001	APS	APS	-	DRB1*03			-	-		
	- aCL IgM	MAS	5.10 [1.39-18.74]	0.008	APS	APS	DRB1*13	-			DRB1*07	-		
	- aβ2GPI IgG	MAS	11.02 [1.38-88.19]	0.006	APS	APS	DRB1*10 DRB1*13	-			-	-		
	- aβ2GPI IgM	MAS	5.92 [1.87-18.73]	0.001	APS	APS	DRB1*13	DRB1*08			-	-		
Genetic	Class II HLA & PTPN22	(n.s.)												

The analysis of confounding factors was accomplished through logistic regression; the table presents the confounding factors that had statistical significance (under "Which") and the variable with highest influence (under "favouring"). (aPL=antiphospholipid antibodies; aCL=anticardiolipin antibodies; aβ2GPI= anti-β2 glycoprotein I antibodies)

Antiphospholipid syndrome

The APS group's distribution is represented in Figure 5.

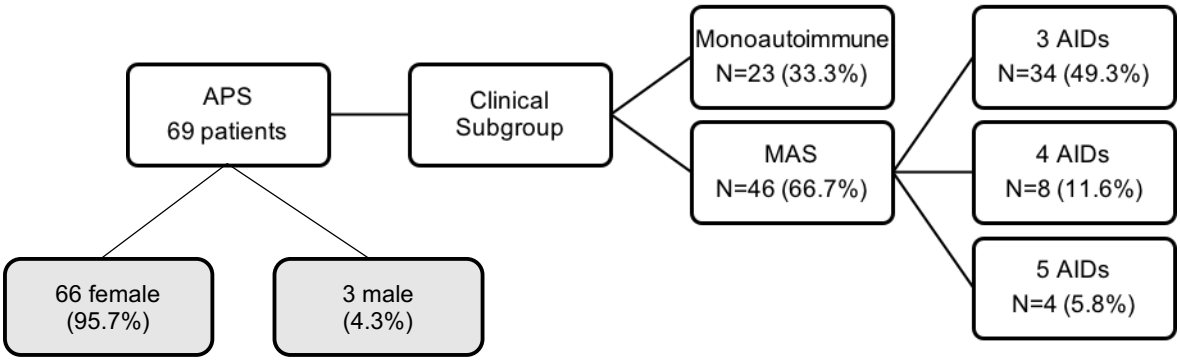

Figure 5: Characterization of APS group in the study cohort
(MAS=multiple autoimmune syndrome; AID=autoimmune disease)

The mean age at onset of APS symptoms was 34.70y (SD 13.41, span 7-75) and at APS diagnosis 37.38y (SD 12.85; span 7-75yo); mean follow-up time was 12.65y (SD 6.85, span 2-41y).

In Table X are the results for the HLA class II allelic frequencies in the APS group and its mono and MAS subgroups. In Table XI the allelic frequencies for PTPN22 (rs2476601) polymorphism, compared to those of the control population.

Table X: HLA class II allelic frequencies in control population and antiphospholipid syndrome study group

Class II HLA	Control population	APS group								
		All			Mono			MAS		
	%	%	OR	p	%	OR	p	%	OR	p
DRB1*01	23.40	14.75	-	n.s.	10.00	-	n.s.	17.07	-	n.s.
DRB1*03	15.60	**39.34**	**3.51**	**<0.001**	30.00	-	n.s.	**43.90**	**4.23**	**<0.001**
DRB1*04	24.47%	27.87	-	n.s.	40.00	-	n.s.	21.95	-	n.s.
DRB1*07	25.53%	19.67	-	n.s.	25.00	-	n.s.	17.07	-	n.s.
DRB1*08	8.51%	8.20	-	n.s.	0.00	-	n.s.	12.22	-	n.s.
DRB1*09	4.96%	0.00	-	n.s.	0.00	-	n.s.	0.00	-	n.s.
DRB1*10	3.90%	3.28	-	n.s.	5.00	-	n.s.	2.44	-	n.s.
DRB1*11	19.50%	14.75	-	n.s.	20.00	-	n.s.	12.20	-	n.s.
DRB1*12	3.19%	1.64	-	n.s.	0.00	-	n.s.	2.44	-	n.s.
DRB1*13	29.79%	18.03	-	n.s.	20.00	-	n.s.	17.07	-	n.s.
DRB1*14	6.03%	6.56	-	n.s.	10.00	-	n.s.	4.88	-	n.s.
DRB1*15	19.90%	18.03	-	n.s.	10.00	-	n.s.	21.95	-	n.s.
DRB1*16	4.61%	9.84	-	n.s.	0.00	-	n.s.	**14.63**	**3.55**	**0.011**

Table XI: PTPN22 allelic frequencies in control population and antiphospholipid syndrome study group

PTPN22 alleles	Control population	APS group								
		All			Mono			MAS		
	%	%	OR	p	%	OR	p	%	OR	p
C	93.1	82.5	0.35	<0.001	84.6	0.41	<0.001	81.1	0.32	<0.001
T	6.9	17.5	2.86		15.4	2.46		18.9	3.14	

In the APS group, the comparison between monoautoimmune and MAS patients yielded some differences, presented in Table XII. There were no significant differences in genetic polymorphisms.

Analysing potential confounding factors, only anti-U1RNP and rheumatoid factor can be attributed solely to MAS. Anti-dsDNA is mostly due to MAS, but SLE is also influential. Raynaud's phenomenon is due to SLE co-occurrence, though MAS retains influence. Anti-Ro/SSA is mostly due to coexisting SjS, but MAS is also influential; anti-La/SSB is solely due to SjS. Anti-Sm and low C3 and C4 are solely due to coexisting SLE.

Given the found differences, we aimed to understand if some immunologic and/or genetic variable could account for them – Table XII also presents these possible risk (positively associated) and protection (negatively associated) factors. No significant associations were found in the monoautoimmune subgroup.

The statistical values for these and other associations can be found in Appendix 5, Tables XXVII-XXXI.

Table XII: APS group significant differences between mono and MAS subgroups, with corresponding confounding factors and risk/protection genetic and immunologic factors

Clinical, immunologic and genetic variables		APS Mono vs. MAS			Confounding factors		MAS APS				Mono APS			
		favouring	OR [95%CI]	p	Which	favouring	Genetic		Immunologic		Genetic		Immunologic	
							Risk	Protection	Risk	Protection	Risk	Protection	Risk	Protection
Organ involvement	Non-thrombotic - global	MAS	4.69 [1.31-16.71]	0.020	-	-	-	-	aCL IgG & IgM	-	-	-	-	-
	Other - Raynaud's phenomenon	MAS	9.12 [2.37-35.19]	<0001	SLE	SLE & MAS	-	-	aβ2GPI IgM	-	-	-	-	-
Antibodies	anti-dsDNA	MAS	5.11 [2.85-9.18]	<0.001	SLE	MAS	-	DRB1*07			-	-		
	low C3	MAS	5.60 [1.88-16.69]	0.001	SLE	SLE	-	DRB1*07 DRB1*11			-	-		
	low C4	MAS	8.23 [2.66-25.51]	<0.001	SLE	SLE	-	DRB1*07			-	-		
	anti-Ro/SSA	MAS	2.14 [1.57-2.93]	<0.001	SjS	SjS>MAS	-	DRB1*08			-	-		
	anti-La/SSB	MAS	1.36 [1.14-1.63]	0.006	SjS	SjS	DRB1*03	-			-	-		
	anti-Sm	MAS	1.25 [1.08-1.45]	0.025	SLE	SLE	DRB1*08	-			-	-		
	anti-U1RNP	MAS	1.61 [1.28-2.02]	0.001	-	MAS	-	DRB1*13			-	-		
	rheumatoid factor	MAS	4.69 [0.96-22.93]	0.042	-	MAS	DRB1*11	-			-	-		
Genetics	Class II HLA & PTPN22	(n.s.)												

The analysis of confounding factors was accomplished through logistic regression; the table presents the confounding factors that had statistical significance (under "Which") and the variable with highest influence (under "favouring"). (aCL=anticardiolipin antibodies; aβ2GPI= anti-β2 glycoprotein I antibodies)

Triple Positive MAS

The Triple Positive MAS group's distribution is represented in Figure 6.

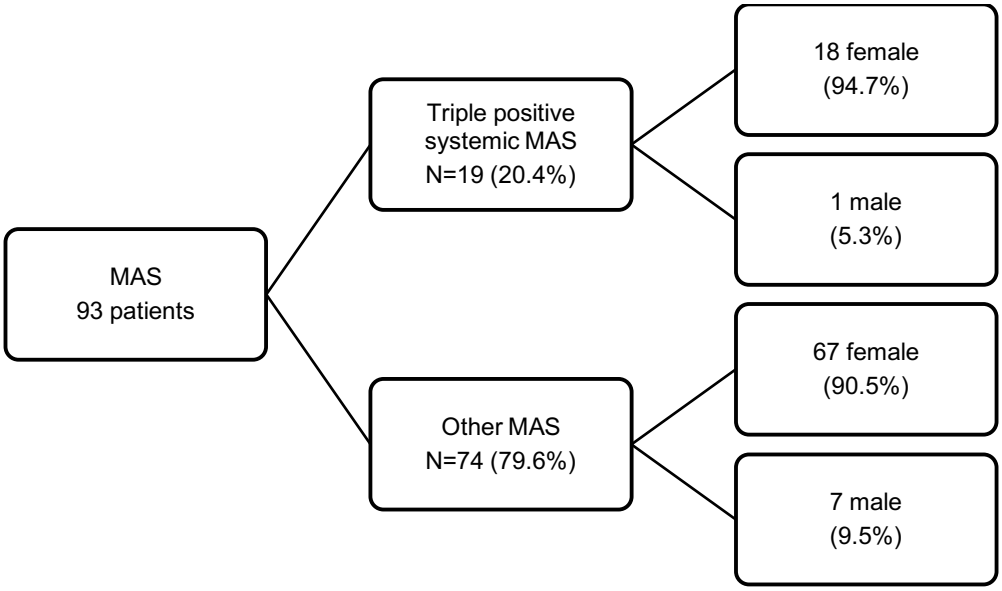

Figure 6: Characterization of MAS subgroup in the study cohort
(MAS=multiple autoimmune syndrome; AID=autoimmune disease; triple positive systemic MAS=SLE+SjS+APS)

The HLA class II allelic frequencies for the MAS group and its triple positive systemic MAS and other MAS subgroups, compared to those of the control population, are shown in Table XIII; the allelic frequencies for PTPN22 (rs2476601) polymorphism for the same groups are presented in Table XIV.

Table XIII: HLA class II allelic frequencies in control population and MAS study group

Class II HLA	Control population	MAS group								
		All			Triple + MAS			Other MAS		
	%	%	OR	p	%	OR	p	%	OR	p
DRB1*01	23.40	18.07	-	n.s.	5.56	-	n.s.	21.54	-	n.s.
DRB1*03	15.60	**44.60**	**4.35**	**<0.001**	**55.60**	**6.76**	**<0.001**	**41.50**	**3.84**	**<0.001**
DRB1*04	24.47	19.28	-	n.s.	33.33	-	n.s.	15.38	-	n.s.
DRB1*07	25.53	19.28	-	n.s.	5.56	-	n.s.	23.08	-	n.s.
DRB1*08	8.51	13.25	-	n.s.	5.56	-	n.s.	15.38	-	n.s.
DRB1*09	4.96	0.00	-	**0.039**	0.00	-	n.s.	0.00	-	n.s.
DRB1*10	3.90	2.41	-	n.s.	5.56	-	n.s.	1.54	-	n.s.
DRB1*11	19.50	**8.43**	**0.04**	**0.018**	16.67	-	n.s.	**6.15**	**0.27**	**0.010**
DRB1*12	3.19	1.20	-	n.s.	0.00	-	n.s.	1.54	-	n.s.
DRB1*13	29.79	19.28	-	n.s.	27.78	-	n.s.	**16.92**	**0.48**	**0.036**
DRB1*14	6.03	2.41	-	n.s.	5.56	-	n.s.	1.54	-	n.s.
DRB1*15	19.90	24.10	-	n.s.	22.22	-	n.s.	24.62	-	n.s.
DRB1*16	4.61	**10.84**	**2.52**	**0.036**	11.11	-	n.s.	10.77	-	n.s.

Table XIV: PTPN22 allelic frequencies in control population and MAS study group

PTPN22 alleles	Control population	MAS group								
		All			Triple + MAS			MAS		
	%	%	OR	p	%	OR	p	%	OR	p
C	93.1	83.9	0.39	<0.001	92.9	-	n.s.	82.1	0.34	<0.001
T	6.9	16.1	2.59		7.1	-		17.9	2.94	

The comparison between the two groups yielded some results, as shown in Table XV. There were no significant associations found between genetic or immunologic factors and clinical variables.

Given the found differences, we aimed to understand if some immunologic and/or genetic variable could account for them. Therefore, Table XV also presents these possible risk (positively associated) and protection (negatively associated) factors.

Table XV: MAS group significant differences between triple positive systemic MAS and other MAS subgroups, with corresponding confounding factors and risk/protection genetic and immunologic factors

Clinical, immunologic and genetic variables		Triple positive systemic MAS vs. other MAS			Triple positive systemic MAS				Other MAS			
		favouring	OR [95%CI]	p	Genetic		Immunologic		Genetic		Immunologic	
					Risk	Protection	Risk	Protection	Risk	Protection	Risk	Protection
Organ involvement	Renal - severe	Triple positive	11.67 [1.49-91.54]	0.021	-	-	-	-	-	-	-	-
	Thrombotic manifestations - CNS thrombosis	Triple positive	4.44 [1.39-14.17]	0.009	-	-	-	-	-	-	-	-
Antibodies	anti-La/SSB	Triple positive	5.32 [1.82-15.55]	0.001	-	DRB1*04			DRB1*03	-		
	aβ2GPI IgG	Triple positive	5.21 [1.77-15.37]	0.005	-	-			-	-		
	aβ2GPI IgM	Triple positive	3.25 [1.15-9.18]	0.022	-	-			DRB1*13	DRB1*03		
	LAC	Triple positive	6.30 [1.58-25.08]	0.009	-	-			-	-		
Genetics	Class II HLA & PTPN22	(n.s.)										

(CNS=central nervous system; aβ2GPI= anti-β2 glycoprotein I antibodies; LAC=lupus anticoagulant)

PTPN22

In the study cohort, PTPN22_T was significantly more frequent than in the control population, in all groups and subgroups (except in monoautoimmune SjS and triple positive systemic MAS). It was a risk factor for the expression of anti-dsDNA and a protection factor against anti-La/SSB expression. In the SLE group, PTPN22_T was protective against aCL IgM expression in the MAS subgroup and against LAC in the global group. On the other hand, it was a risk factor for gastrointestinal involvement in the monoautoimmunity subgroup. In the SjS study group, PTPN22_T had a protective role against anti-Ro/SSA expression in the monoautoimmunity subgroup. It was, however, a risk factor for pulmonary involvement in the SjS group, a risk for low C3 and anti-CCP in the MAS subgroup and a risk for anti-peroxidase expression in the mono subgroup. In the APS group, PTPN22_T had a protective role against aCL IgM; a protective role against LAC in the MAS subgroup, but these were likely associated with SLE and not APS.

Follow-up time

Considering the clinical differences found in our groups, we divided the patients in subgroups of 5 follow-up-years to determine when divergences start.

SLE group:

- NPSLE: more frequent in the MAS subgroup only from the second follow-up tier (5-9 years) onwards (statistically significant); in patients with less than 5 years follow-up, NPSLE was more frequent in the mono subgroup (not statistically significant). The same happens for central focal and central diffuse NPSLE;
- Mucocutaneous subacute: more frequent only in MAS patients with 5 or more years; in the first tier (< 5 years), the mono subgroup had a higher frequency of cases (not statistically significant);
- Musculoskeletal muscle / tendon: this type of involvement was always more frequent in the MAS subgroup (statistically significant only after 10 years of follow-up);
- Haematological: this involvement (in general and thrombocytopenia in particular) was always more frequent in the MAS subgroup
- Raynaud phenomenon: in patients with less than 5 years of follow-up, it was more frequent in the mono subgroup; after that, it was more frequent in the MAS subgroup (only statistically significant after 10 years of follow-up).

SjS group:

- Parotid enlargement: more frequent in the monoautoimmune subgroup across follow-up tiers;
- Cryoglobulins: in patients with less than 5 years follow-up, it is more frequent in the monoautoimmune subgroup (not statistically significant); in patients with more than 5 years follow-up it is more frequent in the MAS;
- Low complement: globally more frequent in the MAS subgroup; however, in patients with 10-14 years and more than 20 years follow-up it is more frequent in the mono subgroup (not statistically significant);
- Raynaud's phenomenon: always more prevalent in the MAS subgroup.

APS group:

- Non-thrombotic: global – in patients with less than 5 years follow-up the frequency is similar in both subgroups; after that, it is more frequent in MAS; Raynaud's phenomenon – more frequent in MAS across all tiers.

Discussion

Our study confirmed HLA-DRB1*03 as an important risk factor for AIDs, namely SLE and SjS. It also confirmed a recent finding that HLA-DRB1*13 could have a protective role in autoimmunity[12] and adds a possible association with HLA-DRB1*14 allele also as a protective factor. Interestingly, HLA-DRB1*13 and *14 alleles have structural similarities and constitute the former DR6 serologically defined group.

HLA-DRB1*11 has been reported as a possible protective factor for SLE in a population from Latin America.[6] In our European population it was a protector against AIDs in general and also a protective factor for SjS, but not statistically significant in monoautoimmune SLE. HLA-DRB1*01 allelic frequency was also significantly lower in our study cohort. Other authors suggested that this allele could have a protective role against SjS[36] but we did not find significant differences in SjS mono subgroup. HLA-DRB1*09 frequency in our cohort is very low when compared to the control population; however, that should be interpreted cautiously, as HLA-DRB1*09 frequency in our control population is significantly higher than the one reported in other cohorts from the same geographic area.[55]

In the SLE group, the HLA-DRB1*16 allele has a higher frequency but only in the MAS subgroup. Even though we did not find an association between this allele and SjS, it has been cited as a potential risk factor for SjS[36], which could justify the increased allele

frequency in the SLE MAS subgroup. The fact that this allele was similarly a risk factor for anti-Ro/SSA (also in the SLE group) reinforces that possibility.
In the SjS group, HLA-DRB1*15 has an increased frequency and could, therefore, be considered a risk factor. The role of this allele in primary SjS has already been reported[56] and this is in accordance with our observations in the monoautoimmune subgroup. It should be noted that HLA-DRB1*15 and *16 alleles also have structural similarities and constitute the former DR2 serologically defined group.
Concerning APS, our study does not confirm the reported risk of HLA-DRB1*04 and *07[34,57-59] and does not find any other significant risk or protection factors.

Despite confirming the possible protective role of HLA-DRB1*13 in AIDs, our study found an association between this allele and antiphospholipid antibodies in MAS patients (aβ2GPI IgM in MAS SLE, aCL IgM, aβ2GPI IgG and IgM in MAS SjS and aβ2GPI in other MAS). Other studies found similar associations, particularly in SLE patients[60-61], but none as broad in spectrum as ours. This could point to HLA-DRB1*13 as a marker for vascular risk in patients with MAS.

Several studies dwelled on the subject of AID co-occurrence.[62-66] Nevertheless, so far there is no consensus whether polyautoimmunity has a significant impact on disease severity, with some studies reporting a more severe disease course[67-69] while others conclude it has a protective effect.[70-73] Overall, in our study the MAS subgroups had more severe organ involvement than the monoautoimmunity subgroups. It could reasonably be argued that the dispersion of immunologic attacks inherent to polyautoimmunity would result in a milder disease course – however, that does not seem to be the case. Although we found no significant differences in several major areas (renal and cardiorespiratory in SLE, thrombotic events in APS), the differences we did find indicate that the coexistence of AIDs has a synergic effect, leading to more severe disease manifestations and evolution, especially for SLE and SjS.

Anti-U1RNP is mentioned by some authors as a risk factor for polyautoimmunity,[27] and by some publications as a protective factor against it.[27, 65-66] In our study it seems to be a risk factor for MAS. It also was, across all groups, the one antibody consistently associated with MAS. Although in the SLE group it was mostly due to coexisting SjS and in the SjS group mostly due to coexisting SLE, MAS retained influence in both groups and was the sole responsible for anti-U1RNP in MAS APS subgroup. It could be argued that anti-U1RNP could be a marker for connective tissue diseases and could be used to ascertain probability of developing a second connective tissue disease on patients with

an established AID. Some studies found an association between prevalence of sicca symptoms and anti-U1RNP titers[74]. Although there could be a bias since we didn't study the differences between high and low anti-U1RNP positive titers, anti-U1RNP positivity seemed to have a protective role against sicca, contradicting those studies.
HLA-DRB1*15 allele, a risk factor for SjS in our study, was also found to be a risk factor for anti-U1RNP.

Systemic lupus erythematosus
HLA-DRB1*07 allele frequency was significantly lower in the MAS subgroup, suggesting a protective role against multiautoimmunity.
The role of antibody expression in NPSLE, although extensively studied, has controversial results.[75] Studies in antiphospholipid antibodies, particularly aCL and LAC, diverge in their findings, denying or reporting a positive association.[75] The role of aβ2GPI is much less studied[75] and results find no association between such antibodies expression and NPSLE. Our study confirms the association of aPL and aCL with NPSLE in general and with central focal involvement in particular; and contrary to previous studies,[75] we found a positive association between aβ2GPI (although never in the monoautoimmune subgroup) and NPSLE in general and with central focal and diffuse in particular. No association with LAC was found. The heightened frequency of aPLs expression in MAS patients could represent a population in the spectrum of APS, but lacking overt clinical thrombotic criteria.
In the MAS subgroup, no genetic or immunologic risk or protective factors were found for NPSLE. However, there was a significant positive association between HLA-DRB1*04 and the expression of anti-β2Glycoprotein I IgG, an antibody with a clear risk role in NPSLE in the SLE group.
Recent studies have found an association between anti-U1RNP antibodies in cerebrospinal fluid and NPSLE, despite a lack of association with antibody serum levels.[76-78] In our study, however, we found serum anti-U1RNP to have a protective role against central focal NPSLE in the monoautoimmunity subgroup.
Authors have described associations of anti-Sm antibodies with organ involvement and disease activity, reporting mixed results also involving NPSLE.[79] In our study, anti-Sm had a protective role in NPSLE, in the global and mono-SLE groups.
There is a generally accepted association between anti-Ro/SSA antibodies and subacute cutaneous SLE.[65] Our study confirms this positive association in our SLE group (global and MAS subgroup). In the MAS subgroup, HLA-DRB1*16 was a risk factor for anti-Ro/SSA expression and both were risk factors for subacute lesions. As

mentioned above, HLA-DRB1*16 is considered by some authors as a possible risk factor for SjS, which might corroborate our findings.

Several studies dwell on the effect of anti-C1q on renal involvement in SLE[80]; however, to the best of our knowledge, no study mentions an association between this antibody and subacute lesions or NPSLE. In our study, anti-C1q was a susceptibility factor for the former and a protective factor against the latter.

Sjögren's syndrome

We found a positive association between anti-centromere antibody and cryoglobulinemia in the MAS subgroup. To the best of our knowledge, it is the first time such an association has been described. There is a clear association between anti-centromere and Raynaud's phenomenon[81-82], which we did not find. This could be due to the low number of patients with positive anti-centromere antibody or to the fact that Raynaud's phenomenon is transversal to AIDs, with multiple possible causes.

We also found an association between Raynaud's phenomenon and anti-U1RNP antibodies, a relationship previously established in connective tissue diseases, particularly MCTD[83-85] but also in SLE patients[86].

Triple positive systemic MAS

Patients with SLE, SjS and APS seem to be at greater risk of developing severe lupus nephritis than other MAS patients. It is possible that these events are not totally independent, as aPLs and thrombotic events can contribute greatly to aggravate lupus nephritis[87]. Simultaneously, they seem to have a greater risk of thrombotic events, namely CNS thrombosis. The triple positive systemic MAS might represent a more severe pro-thrombotic state. However, these are all conjectures, as this is, to the best of our knowledge, the first study to dwell on this specific AID association.

Follow-up time

There is no consensus about how much follow-up time is needed to clearly differentiate pure monoautoimmune patients from those who could become polyautoimmune. It is often difficult to determine how much time spans between the beginning of distinct AIDs in one single patient, due to delayed diagnosis and common subphenotypes in various AIDs. These juxtapositions frequently lead to erroneous attribution of new symptoms to a previously established AID, instead of hypothesizing the emergence of a new one. The consequent overestimation of time of disease onset should be considered as a bias in retrospective studies as this one (and in polyautoimmunity studies in general).

Incomplete medical files also constitute a problem for accurately establishing a timeline of disease(s) evolution.

Despite all this, our findings allow us to conclude that the difference between the pure monoautoimmune patients and the MAS patients might arise between 5 and 10 years after diagnosis.

PTPN22

Several studies focused on determining the role of PTPN22 as a possible susceptibility factor for AIDs.[1,15,39-48,88-89] Some suggest a protective role against infectious diseases[90-91] and others found a polymorphism with a possible protective role against AIDs[43]. However, and to the best of our knowledge, this seems to be the first study that finds its (rs2476601) polymorphism to be associated with a less severe disease course. In our cohort, it had a protective role against aCL IgM, LAC and anti-Ro/SSA expression, which could translate into a more benign prognosis in some patients. Despite this, it was also a susceptibility factor for some types of organ involvement and more studies are necessary to clarity its role.

Conclusion

To the best of our knowledge, this is one of the few studies to focus on all three nosologic dimensions of MAS: organ involvement, immunologic expression and genetic polymorphisms. We concluded that the coexistence of several AIDs contributes to a more severe disease course. This might not be easily apparent to clinicians due to the fact that several AIDs are treated by different doctors (especially when non-systemic AIDs coexist with systemic AIDs), leading to absence of an integrated picture of each patient.

We confirmed several previously established associations between genetic polymorphisms, fortifying their definition as autoimmune risk or protection factors. We believe HLA-DRB1*07 could be used as a marker for monoautoimmunity, thus allowing to stratify patients with one AID in terms of probability of developing additional AIDs. Similarly, anti-U1RNP could also be used as an immunologic marker for development of a second connective tissue disease in patients with an established AID.

The positive association of HLA-DRB1*13 with antiphospholipid antibodies in MAS patients could lead to use of this allele as a predictive tool for vascular risk in patients with multiple AIDs (without overt APS).

The role of PTPN22 (rs2476601) polymorphism in autoimmunity is established but not totally understood. Even though it is clearly more frequent in AID patients, our study

suggests it could be associated with a less severe disease course. This finding opens new possibilities that need confirmation in other cohorts.

The coexistence of the three systemic chaperones of autoimmunity seems to create a pro-thrombotic state, with higher risk of CNS thrombosis and severe renal involvement. Further studies are necessary to fully understand this apparent synergic relationship and its implications.

Finally, we suggest that the divergence between monoautoimmune and multiple autoimmune syndrome patients arises 5 to 10 years after initial diagnosis. This conclusion could be important in preventing future study biases – to adequately compare mono and multiple autoimmune patients, it could be advisable to select a population with more than 5 years of diagnosis.

Although we had many significant associations, most of them were exploratory at best and need to be confirmed in bigger cohorts and in patients with different ethnical ascendancies.

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Bibliography

[1] Criswell, L., Pfeiffer, K., Lum, R., Gonzales, B., Novitzke, J., Kern, M., Moser, K., Begovich, A., Carlton, V., Li, W., Lee, A., Ortmann, W., Behrens, T. & Gregersen, P. (2005) Analysis of families in the multiple autoimmune disease genetics consortium collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. *Am J Hum Genet*. 2005 Apr;76(4):561-71.

[2] Anaya, J-M., Gómes, L. & Castiblanco, J. (2006) Is there a common genetic basis for autoimmune diseases? *Clin Dev Immunol*. 2006 Jun-Dec;13(2-4):185-95.

[3] Anaya, J-M., Corena, R., Castiblanco, J., Rojas-Villarraga, A. & Shoenfeld, Y. (2007) The kaleidoscope of autoimmunity: multiple autoimmune syndromes and familial autoimmunity. *Expert Rev Clin Immunol*. 2007 Jul;3(4):623-35.

[4] Anaya, J-M. (2012) Common mechanisms of autoimmune diseases (the autoimmune tautology). *Autoimmun Rev.* 2012 Sep;11(11):781-4.

[5] Anaya, J-M., Castiblanco, J., Rojas-Villarraga, A., Pineda-Tamayo, R., Levy, R., Gómez-Puerta, J., Dias, C., Mantilla, R., Gallo, J., Cervera, R., Shoenfeld, Y. & Arcos-Burgos, M. (2012) The multiple autoimmune syndromes. A clue for the autoimmune tautology. *Clin Rev Allergy Immunol* 2012 Dec;43(3):256-64.

[6] Cruz-Tapias, C., Pérez-Fernández, O., Rojas-Villarraga, A., Rodríguez-Rodríguez, A., Arango, M-T & Anaya, J-M.(2012) Shared HLA Class II in six autoimmune diseases in Latin America: a meta-analysis. *Autoimmune Dis.* 2012;2012:569728.

[7] Amador-Patarroyo, M., Arbelaez, J., Mantilla, R., Rodriguez-Rodriguez, A., Cárdenas-Roldán, J., Pineda-Tamayo, R., Guarin, M., Kleine, L., Rojas-Villarraga, A. & Anaya, J-M. (2012) Sjogren's syndrome at the crossroad of polyautoimmunity. *J Autoimm*. 2012 Sep;39(3):199-205.

[8] Anaya, J-M., Rojas-Villarraga, A. & García-Carrasco, M. (2012) The autoimmune tautology: from polyautoimmunity and familial autoimmunity to the autoimmune genes. *Autoimmune Dis*. 2012;2012:297193.

[9] Cárdenas-Roldán, J., Rojas-Villarraga, A. & Anaya, J-M. (2013) How do autoimmune diseases cluster in families? A systematic review and meta-analysis. *BMC Med*. 2013 Mar 18;11:73.

[10] Perricone, C., Agmon-Levin, N., Ceccarelli, F., Valesini, G., Anaya, J-M. & Shoenfeld, Y. (2013) Genetics and autoantibodies. *Immunol Res* 2013 Jul;56(2-3): 206-19.

[11] Franco, J-S. & Anaya, J-M. (2014) The autoimmune tautology with a focus on antiphospholipid syndrome. *Lupus*. 2014 Oct;23(12):1273-5.

[12] Bettencourt, A., Carvalho, C., Leal, B., Brás, S. Lopes, D., Silva, A., Santos, E., Torres, T., Almeida, I., Farinha, F., Barbosa, P., Marinho, A., Selores, M., Correia, J. Vasconcelos, C., Costa, P. & Martins da Silva, B. (2015) The protective role of HLA-DRB1*13 in autoimmune disease. *J Immunol Res*. 2015;2015:948723.

[13] Betterle, C., Garelli, S., Coco, G. & Burra, P. (2014) A rare combination of type 3 autoimmune polyendocrine syndrome (APS-3) or multiple autoimmune syndrome (MAS-3). *Auto Immun Highlights*. 2014 Feb 11;5(1):27-31.

[14] Tobón, G., Pers, J-O., Cañas, C., Rojas-Villarraga, A., Youinou, P. & Anaya, J-M. (2012) Are autoimmune diseases predictable? *Autoimmunity Reviews* 11 (2012) 259-266.

[15] Eliopoulos, E., Zervou, M., Andreou, A., Dimopoulou, K., Cosmidis, N., Voloudakis, G., Mysirlaki, H., Vazgiourakis, V., Sidiropoulos, P., Niewold, T., Boumpas, D. & Goulielmos, G. (2011) Association of the PTPN22 R620W polymorphism with increased risk for SLE in the genetically homogeneous population of Crete. *Lupus.* 2011 Apr;20(5):501-6.

[16] Tozzoli, R., Sorrentino, M. & Bizzaro, N. (2013) Detecting multiple autoantibodies to diagnose autoimmune co-morbidity (multiple autoimmune syndromes and overlap syndromes): a challenge for the autoimmunologist. *Immunol Res*. 2013 Jul;56(2-3):425-31.

[17] Kochi, Y. (2016) Genetics of autoimmune diseases: perspectives from genome-wide association studies. *Int Immunol*. 2016 Apr;28(4):155-61

[18] Somers, E., Thomas, S., Smeeth, L. & Hall, A. (2009) Are individuals with an autoimmune disease at higher risk of a second autoimmune disorder? *Am J Epidemiology*. 2009 Mar 15;169(6):749-55.

[19] Rojas-Villarraga, A., Amaya-Amaya, J., Rodriguez-Rodriguez, A., Mantilla, R. & Anaya, J-M. (2012) Introducing polyautoimmunity: secondary autoimmune diseases no longer exist. *Autoimmune Dis.* 2012:254319.

[20] Cifuentes, R., Restrepo-Montoya, D. & Anaya, J-M. (2012) The autoimmune tautology: an in silico approach. *Autoimmune Dis.* 2012;2012:792106.

[21] Castiblanco, J., Sarmiento-Monroy, J., Mantilla, R., Rojas-Villarraga, A. & Anaya, J-M. (2015) Familial aggregation and segregation analysis in families presenting autoimmunity, polyautoimmunity and multiple autoimmune syndrome. *J Immunol Res.* 2015;2015:572353.

[22] Johar, A., Anaya, J-M., Andrews, D., Patel, H., Field, M., Goodnow, C. & Arcos-Burgos, M. (2015) Candidate gene discovery in autoimmunity by using extreme phenotypes, next generation sequencing and whole exome capture. *Autoimmun Rev.* 2015 Mar;14(3):204-9.

[23] Aggarwal, R., Anaya, J-M., Koelsch, K., Kurien, B. & Scofield, R. (2015) Association between secondary and primary Sjogren's syndrome in a large collection of lupus families. *Autoimmune Dis*. 2015;2015:298506.

[24] Scofield, R., Bruner, G., Harley, J. & Namjou, B. (2007) Autoimmune thyroid disease is associated with a diagnosis of secondary Sjogren's syndrome in familial systemic lupus. *Ann Rheum Dis*. 2007 Mar;66(3): 410-3.

[25] Anaya, J-M. (2014) The diagnosis and clinical significance of polyautoimmunity. *Autoimm Rev*. 2014 Apr-May;13(4-5):423-6.

[26] Johar, A., Mastronardi, C., Rojas-Villarraga, A., Patel, H., Chuah, A., Peng, K., Higgins, A., Milburn, P., Palmer, S., Silva-Lara, M., Velez, J., Andrews, D., Field, M., Huttley, G., Goodnow, C., Anaya, J-M. & Arcos-Burgos, M. (2015) Novel and rare functional genomic variants in multiple autoimmune syndrome and Sjogren's syndrome. *J Transl Med*. 2015 Jun 2;13:173

[27] Rojas-Villarraga, A., Toro, C-E., Espinosa, Gerard, Rodríguez-Velosa, Y., Duarte-Rey, C., Mantilla, R., Iglesias-Gamarra, A., Cervera, R. & Anaya, J-M. (2010) Factors influencing polyautoimmunity in systemic lupus erythematosus. *Autoimmun Rev*. 2010 Feb;9(4):229-32.

[28] Szyper-Kravitz, M. Marai, I. & Shoenfeld, Y. (2005) Coexistence of thyroid autoimmunity with other autoimmune diseases: friend or foe? Additional aspects on the mosaic of autoimmunity. *Autoimmunity*. 2005 May;38(3):247-55.

[29] Castiblanco, J. & Anaya, J-M. (2007) The nature and nurture of common autoimmunity. *Ann N Y Acad Sci*. 2007 Aug;1109:1-8.

[30] Cooper, G., Byrum, M. & Somers, E. (2009) Recent insights in the epidemiology of autoimmune diseases: improved prevalence and understanding of clustering of diseases. *J Autoimmun*. 2009 Nov-Dec;33(3-4):197-207.

[31] Tanimura, K., Jin, H., Suenaga, T., Morikami, S., Arase, N., Kishida, K., Hirayasu, K., Kohyama, M., Ebina, Y., Yasuda, S., Horita,
T., Takasugi, K., Ohmura, K., Yamamoto K., Katamaya, I., Sasazuki, T., Lanier, L., Atsumi, T., Yamada, H. & Arase, H. (2015) B2-glycoproteinI/HLA class II complexes are novel autoantigens in antiphospholipid syndrome. *Blood*. 2015 Apr 30;125(18):2835-44.

[32] Arase, N. & Arase, H. (2015) Cellular misfolded proteins rescued from degradation by MHC class II molecules are possible targets for autoimmune diseases. *J Biochem*. 2015 Nov;158(5):367-72.

[33] Camps MT., Cuadrado, MJ, Ocón, P., Alonso, A., Gutierrez, A., Guil, M., Grana, MI & Ramón, E. (1995) Association between HLA class II antigens and primary antiphospholipid syndrome from the south of Spain. *Lupus*. 1995 Feb;4(1):51-5.

[34] Bertolaccini, M., Atsumi, T., Caliz, A., Amengual, O., Khamashta, M., Hughes, G. & Koike, T. (2000) Association of antiphosphatidylserine/prothrombin autoantibodies with HLA class II genes. *Arthritis Rheum*. 2000 Mar;43(3) 683-8.

[35] Mackay, I. (2009) Clustering and commonalities among autoimmune diseases. *J Autoimmun*. 2009 Nov-Dec;33(3-4):170-7.

[36] Cruz-Tapias, P., Rojas-Villarraga, A., Maier-Moore, S. & Anaya, J-M. (2012) HLA and Sjogren's syndrome susceptibility. A meta-analysis of worldwide studies. *Autoimmun Rev.* 2012 Feb;11(4):281-7.

[37] Kamboh, M., Wang, X., Kao, A., Barmada, M., Clarke, A. Ramsey-Goldman, R., Manzi, S. & Demirci, F. (2013) Genome-wide association study of antiphospholipid antibodies. *Autoimmune Dis*. 2013;2013:761046.

[38] Ice, J., Li, H., Adrianto, I., Lin, P., Kelly, J., Montgomery, C., Lessard, C. & Moser, K. (2012) Genetics of Sjogren's syndrome in the genome-wide association era. *J Autoimmun*. 2012 Aug;39(1-2):57-63.

[39] Namjou, B., Kim-Howard, X., Sun, C., Adler, A., Chung, S., Kaufman, K., Kelly, J., Glenn, S., Guthridge, J., Scofield, R., Kimberly, R., Brown, W., Alarcón, G., Edberg, J., Kim, J-H., Choi, J., Ramsey-Goldman, R., Petri, M., Reveille, J., Vilá, L., Boackle, S., Pons-Estel, B., Niewold, T., Sivils, K., Merrill, J., Anaya, J-M., Gilkeson, G., Gaffney, P., Bae, S-C., Alarcón-Riquelme, M., Criswell, L., James, J. & Nath, S. (2013) PTPN22 association in systemic lupus erythematosus with respect to individual ancestry and clinical subphenotypes. *PLoS One* 2013 Aug 7;8(8):e69404.

[40] Salmond, R., Brownlie, R. & Zamoyska, R. (2015) Multifunctional roles of the autoimmune disease-associated tyrosine phosphatase PTPN22 in regulating T cell homeostasis. *Cell Cycle* 2015;14(5):705-11.

[41] Maine, C., Marquardt, K., Scatizzi, J., Pollard, K., Kono, D. & Sherman, L. (2015) The effect of the autoimmunity-associated gene, PTPN22, on the BXSB model of lupus. *Clin Immunol*. 2015 Jan;156(1):65-73.

[42] Nabi, G., Akhter, N., Wahid, M., Bhatia, K., Mandal, R., Dar, S., Jawed, A. & Haque, S. (2016) Meta-analysis reveals PTPN22 Meta-analysis reveals PTPN22 1858C>T polymorphism confers susceptibility to rheumatoid arthritis in Caucasian but not in Asian population. *Autoimmunity*. 2016 May;49(3):197-210.

[43] Machado-Contreras, J., Muñoz-Valle, J., Cruz, A., Salazar-Camarena, D., Marín-Rosales, M. & Palafox-Sánchez, C. (2015) Distribution of PTPN22 polymorphisms in SLE from western Mexico: correlation with mRNA expression and disease activity. *Clin Exp Med* 2015 May 27.

[44] Sharp, R., Abdulrahim, M., Naser, E. & Naser, S. (2015) Genetic variations of PTPN2 and PTPN22: role in the pathogenesis of type 1 diabetes and Crohn's disease. *Front Cell Infect Microbiol*. 2015 Dec 24;5:95.

[45] Piotrowski, P., Lianeri, M., Wudarski, M., Lacki, J. & Jagodzinski, P. (2008) Contribution of the R620W polymorphism of protein tyrosine phosphatase non-receptor 22 to systemic lupus erythematosus in Poland. *Clin Exp Rheumatol.* 2008 Nov-Dec;26(6):1099-102.

[46] Stanford, S. & Bottini, N. (2014) PTPN22: the archetypal non-HLA autoimmunity gene. *Nat Rev Rheumatol*. 2014 Oct;10(10):602-611.

[47] Rodríguez, G. & Zéron, H. (2015) Familial autoimmune thyroid disease and PTPN22. *Medicinski Glasnik*, vol 12, number 2, August 2015.

[48] Tavares, N., Santos, M., Moura, R., Araújo, J., Guimarães, R., Crovella, S. & Brandão, L. (2015) Association of TNF-α and PTPN22 polymorphisms with type 1 diabetes and other autoimmune diseases in Brazil. *Genet Mol Res* 2015 Dec 28;14(4):18936-44.

[49] Hochbert, M. (1997) Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 1997 Sep;40(9):1725.

[50] Shiboski, S., Shiboski, C., Criswell, I., Baer, A., Challacombe, S., Lanfrenchi, H., Schiødt, M., Umehara, H., Vivino, F., Zhao, Y., Dong, Y., Greenspan, D., Heindereich, A., Helin, P., Kirkham, B., Katagawa, K., Larkin, G., Li, M., Lietman, T., Lindegaard, J., McNamara, N., Sack, K., Shirlaw, P., Sugai, S., Vollenweider, C., Whitcher, J., Wu, A., Zhang, S., Zhang, W., Greenspan, J. & Daniels, T. (2012) American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's international collaborative clinical alliance cohort. *Arthritis Care Res* (Hoboken). 2012 Apr;64(4):475-87.

[51] Garg, N. & Deodhar, A. (2012) The Sydney classification criteria for definite antiphospholipid syndrome. *J Musculoskel Med*. 29:73-77.

[52] Hughes, G. (2014) Hughes syndrome/APS. 30 years on, what have we learned? Opening talk at the 14th international congress on antiphospholipid antibodies, Rio de Janeiro, October 2013. *Lupus.* 2014 Apr;23(4):400-6.

[53] Miller, S., Dykes, D. & Polesky, H. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988 Feb 11;16(3):1215.

[54] Olerup, O. & Zetterquist, H. (1992) HLA-DR typing by PCR amplification with sequence – specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens*. 1992 May;39(5):225-35.

[55] Lima, B. & Alves, H.(2013) HLA-A, -C, -B and –DRB1 allelic and haplotypic diversity in bone marrow volunteer donors from northern Portugal. *Organs, tissues & cells* (16), 19-26.

[56] Guggenbuhl, P., Jean, S., Jego, P., Grosbois, B., Chalès, G., Semana, G., Lancien, G., Veillard, E., Pawlotsky, Y. & Perdriger, A. (1998) Primary Sjögren's syndrome: role of the HLA-DRB1*0301-*1501 heterozygotes. *J Rheumatol*. 1998 May;25(5):900-5.

[57] Asherson, R., Fei, H-M., Staub, H., Khamashta, M., Hughes, G. & Fox, R. (1992) Antiphospholipid antibodies and HLA associations in primary Sjogren's syndrome. *Ann Rheum Dis*. 1992 Apr;51(4):495-8.

[58] Sanchez, M., Katsumata, K., Atsumi, T., Romero, F., Bertolaccini, M., Funke, A., Amengual, O., Kondeatis, E., Vaughan, R., Cox, A., Hughes, G. & Khamashta, M. (2004) Association of HLA-DM polymorphism with the production of antiphospholipid antibodies. *Ann Rheum Dis*. 2004 Dec;63(12):1645-8.

[59] Freitas, M., Silva, L., Deghaide, N., Donadi, E. & Louzada-Júnior, P. (2004) Is HLA class II susceptibility to primary antiphospholipid syndrome different from susceptibility to secondary antiphospholipid syndrome? *Lupus.* 2004; 13(2):125-31.

[60] Lundström, E., Gustafsson, J., Jönsen, A., Leonard, D., Zickert, A., Elvin, K., Sturfelt, G., Nordmark, G., Bengtsson, A., Sundin, U., Källberg, H., Sandling, J., Syvänen, A., Klareskog, L., Gunnarsson, I., Rönnblom, L., Padyukov, L. & Svenungsson, E. (2013) HLA-DRB1*04/*13 alleles are associated with vascular disease and antiphospholipid antibodies in systemic lupus erythematosus. *Ann Rheum Dis*. 2013 Jun;72(6):1018-25.

[61] Kapitany, A., Tarr, T., Gyetvai, A., Szodoray, P., Tumpek, J., Poor, G., Szegedi, G., Sipka, S., Kiss, E. (2009) Human leukocyte antigen-DRB1 and –DQB1 genotyping in lupus patients with and without antiphospholipid syndrome. *Ann N Y Acad Sci*. 2009 Sep;1173:545-51.

[62] McDonagh, J. & Isenberg, D. (2000) Development of additional autoimmune diseases in a population of patients with systemic lupus erythematosus. *Ann Rheum Dis*. 2000 Mar;59(3) 230-2.

[63] Lazarus, M. & Isenberg, D. (2005) Development of additional autoimmune diseases in a population of patients with primary Sjogren's syndrome. *Ann Rheum Dis*. 2005 Jul;64(7):1062-4

[64] Theander, E. & Jacobsson, L. (2008) Relationship of Sjogren's syndrome to other connective tissue and autoimmune disorders. *Rheum Dis Clin North Am*.2008 Nov;34(4):935-47.

[65] Ramos-Casals, M., Brito-Zéron, P. & Font, J. (2007) The overlap of Sjogren's syndrome with other systemic autoimmune diseases. *Semin Arthritis Rheum*. 2007 Feb;36(4):246-55.

[66] Baer, A., Maynard, J., Shaikh, F., Magder, L. & Petri, M. (2010) Secondary Sjogren's syndrome in systemic lupus erythematosus defines a distinct disease subset. *J Rheumatol*. 2010 Jun;37(6):1143-9.

[67] Christensen, PB, Jensen, TS, Tsiropoulos, I., Sorensen, T., Kjaer, M., Hojer-Pedersen, E., Rasmussen, MJK & Lehfeldt, E. (1995) Associated autoimmune diseases in myasthenia gravis. A population-based study. *Acta Neurol Scand* 1995 Mar;91(3): 192-5.

[68] Valerio, G., Maiuri, L., Troncone, R., Buono, P., Lombardi, F., Palmieri, R. & Franzese, A. (2002) Severe clinical onset of diabetes and increased prevalence of other autoimmune diseases in children with coeliac disease diagnosed before diabetes mellitus. *Diabetologia.* 2002 Dec;45(12):1719-22.

[69] Chambers, S., Charman, S., Rahman, A. & Isenberg, D. (2007) Development of additional autoimmune diseases in a multi-ethnic cohort of patients with systemic lupus erythematosus with reference to damage and mortality. *Ann Rheum Dis*. 2007 Sep;66(9):1173-7.

[70] Marinó, M., Ricciardi, R., Pinchera, A., Barbesino, G., Manetti, L., Chiovato, L., Braverman, L., Rossi, B., Muratorio, A. & Mariotti, S. (1997) Mild clinical expression of myasthenia gravis associated with autoimmune thyroid diseases. *J Clin Endocrinol Metab.* 1997 Feb;82(2):438-43.

[71] Manoussakis, M., Georgopoulou, C., Zintzaras, E., Spyropoulou, M., Stavropoulou, A., Skopouli, F. & Moutsopoulos, H. (2004) Sjogren's syndrome associated with systemic lupus erythematosus. Clinical and laboratory profiles and comparison with primary Sjogren's syndrome. *Arthritis Rheum*. 2004 Mar;50(3):882-91.

[72] Avouac, J., Airò, P., Dieude, P., Caramaschi, P., Tiev, K., Diot, E., Sibilia, J., Cappelli, S., Granel, B., Vacca, A, Wipff, J., Meyer, O., Kahan, A., Matucci-Cerinic, M. & Allanore, Y. (2010) Associated autoimmune diseases in systemic sclerosis define a subset of patients with milder disease: results from 2 large cohorts of European Caucasian patients. *J Rheumatol* 2010 Mar; 37(3):608-14.

[73] Xu, D., Tian, X., Zhang, W., Zhang, X, Liu, B. & Zhang, F. (2010) Sjogren's syndrome-onset lupus patients have distinctive clinical manifestations and benign prognosis: a case-control study. *Lupus*. 2010 Feb;19(2):197-200.

[74] Takada, K., Suzuki, K., Matsumoto, M., Okada, M., Nakanishi, T., Horikoshi, H., Higuchi, T. & Ohsuzu, F. (2008) Negative correlation of anti-U1RNP antibody titers and the amount of salivary secretion with age correction. *Intern Med.* 2008;47(5):385-90.

[75] Sciascia, S., Bertolaccini, M., Roccatello, D., Khamashta, A. & Sanna, G. (2014) Autoantibodies involved in neuropsychiatric manifestations associated with systemic lupus erythematosus - a systematic review. *J Neurol*. 2014 Sep;261(9):1706-14.

[76] Okada, J., Hamana, T. & Kondo, H. (2003) Anti-U1RNP antibody and aseptic meningitis in connective tissue diseases. *Scand J Rheumatol*. 2003;32(4):247-52.

[77] Sato, T., Fujii, T., Yokohama, T., Fujita, Y., Imura, Y., Yukawa, N., Kawabata, D., Nojima, T., Ohmura, K., Usui, T. & Mimori, T. (2010) Anti-U1 RNP antibodies in cerebrospinal fluid are associated with central neuropsychiatric manifestations in systemic lupus erythematosus and mixed connective tissue disease. *Arthritis Rheum*. 2010 Dec;62(12):3730-40.

[78] Yokoyama, T., Fujii, T., Kondo-Ishikawa, S., Yamakawa, N., Nakano, M., Yukawa, N., Yoshifuji, H., Ohmura, K. & Mimori, T. (2014) Association between anti-U1 ribonucleoprotein antibodies and inflammatory mediators in cerebrospinal fluid with neuropsychiatric systemic lupus erythematosus. *Lupus*. 2014 Jun;23(7):635-42.

[79] Arroyo-Ávila, M., Santiago-Casas, Y., McGwin Jr. G., Cantor, R., Petri, M., Ramsey-Goldman, R., Reveille, J., Kimberly, R., Alarcón, G., Vilá, L. & Brown, E. (2015) Clinical associations of anti-Smith antibodies in PROFILE: a multi-ethnic lupus cohort. *Clin Rheumatol*. 2015 Jul;34(7):1217-23.

[80] Su, D., Liu, R. Li, X. & Sun, L. (2014) Possible novel biomarkers of organ involvement in systemic lupus erythematosus. *Clin Rheumatol*. 2014 Aug;33(8):1025-31.

[81] Routsias, J. & Tzioufas, A. (2007) Sjögren's syndrome – study of autoantigens and autoantibodies. *Clin Rev Allergy Immunol*. 2007 Jun;32(3):238-51.

[82] Tzioufas, A., Tatouli, I. & Moutsopoulos, H. (2012) Autoantibodies in Sjögren's syndrome: clinical presentation and regulatory mechanisms. *Presse Med* 2012 Sep;41(9 Pt 2):e451-60.

[83] McHugh, N., James, I. & Maddison, P. (1990) Clinical significance of antibodies to a 68kDa U1RNP polypeptide in connective tissue disease. *J Rheumatol*. 1990 Oct;17(10):1320-8.

[84] Rebora, A. & Parodi, A. (1990) Mixed connective tissue disease and correlated diseases. *G Ital Dermatol Venereol*. 1990 Sep;125(9);357-62.

[85] Haustein, U. (2005) MCTD – mixed connective tissue disease. *J Dtsch Dermatol Ges.* 2005 Feb;3(2):97-104.

[86] Furtado, R., Pucinelli, M., Cristo, V., Andrade, L. & Sato, E. (2002) Scleroderma-like nailfold capillaroscopic abnormalities are associated with anti-U1-RNP antibodies and Raynaud's phenomenon in SLE patients. *Lupus*. 2002;11(1):35-41.

[87] Sciascia, S., Baldovino, S., Schreiber, K., Solfietti, L. & Roccatello, D. (2015) Antiphospholipid syndrome and the kidney. *Semin Nephrol*. 2015 Sep;35(5):478-86.

[88] Sanchez, E., Nadig, A., Richardson, B., Freedman, B., Kaufman, K., Kelly, J., Niewold, T., Kamen, D., Gilkeson, G., Ziegler, J., Langefeld, C., Alarcón, G., Edberg, J. Ramsey-Goldman, R., Petri, M., Brown, E., Kimberly, R., Reveille, J., Vilá, L., Merrill, J., Anaya, J-M., James, J., Pons-Estel, B., Martin, J., Park, S-Y., Bang, S-Y., Bae, S-C., Moser, K., Vyse, T., Criswell, L., Gaffney, P., Tsao, B., Jacob, C., Harley, J., Alarcón-Riquelme, M. & Sawalha, A. (2011) Phenotypic associations of genetic susceptibility loci in systemic lupus erythematosus. *Ann Rheum Dis*. 2011 Oct;70(10):1752-1757.

[89] Ivashkiv, L. (2013) PTPN22 in autoimmunity: different cell and different way. *Immunity*. 2013 Jul 25;39(1):91-93.

[90] Lopez-Escarnez, J. (2010) A variant of PTPN22 gene conferring risk to autoimmune diseases may protect against tuberculosis. *J Postgrad Med*. 2010 Jul-Sep;56(3):242-3.

[91] Napolioni, V., Natali, A., Saccucci, P. & Lucarini, N. (2011) PTPN22 1858C>T (R620W) functional polymorphism and human longevity. *Mol Biol Rep*. 2011 Aug;38(6):4231-5.

Appendix 1

Short version of the Cumulative SLE manifestations for Genetic Studies form

Organ involvement

1. Nephrologic Y/N
    a. If yes - type of involvement: 1-5=biopsy grades I-V; 6=biopsy grade VI or renal function replacement techniques; 7=no biopsy but treated for nephritic syndrome; 8=no biopsy but treated for nephrotic syndrome; 9=no biopsy but treated, no record for what
    b. Transplant Y/N; if yes, date.
2. Neuropsychiatric (NPSLE) Y/N
    a. If yes:
        i. Peripheral Y/N
        ii. Central focal Y/N
        iii. Central diffuse Y/N
        iv. Cognitive Y/N
3. Mucocutaneous Y/N
    a. If yes:
        i. Acute Y/N
        ii. Subacute Y/N
        iii. Chronic Y/N
        iv. Vasculitis Y/N
        v. Ulcers Y/N
4. Musculoskeletal Y/N
    a. If yes:
        i. Arthralgia Y/N
        ii. Arthritis Y/N
        iii. Muscle or tendon involvement Y/N
5. Cardiorespiratory Y/N
    a. If yes:
        i. Serositis Y/N
        ii. Endocarditis Y/N
        iii. Coronary arterial disease Y/N
        iv. Pulmonary haemorrhage/vasculitis Y/N
        v. Interstitial alveolitis/pneumonitis Y/N
        vi. Pulmonary hypertension Y/N

6. Hematologic Y/N
    a. If yes:
        i. Haemolytic anaemia Y/N
        ii. Thrombocytopenia Y/N
        iii. Haem phagocytic syndrome Y/N
7. Gastrointestinal Y/N
8. Ophthalmologic Y/N
9. Raynaud's syndrome Y/N
10. Raynaud's related ulcers Y/N

Antibodies

1. anti-dsDNA Y/N
2. anti-C1q Y/N
3. Low C3 Y/N
4. Low C4 Y/N
5. ANAs Y/N
6. anti-ENA Y/N
7. anti-Ro/SSA Y/N
8. anti-La/SSB Y/N
9. anti-alphafodrine Y/N
10. anti-aquaporine 4 Y/N
11. anti-Sm Y/N
12. anti-U1RNP Y/N
13. anti-ribosomal P Y/N
14. anti-nucleosome Y/N
15. anti-CCP Y/N
16. anti-Ro52 Y/N
17. anti-Scl70 Y/N
18. anti-centromere Y/N
19. rheumatoid factor Y/N
20. anti-cardiolipin IgG Y/N
21. anti-cardiolipin IgM Y/N
22. anti-β2glycoprotein I IgG Y/N
23. anti-β2glycoprotein I IgM Y/N
24. anti-prothrombin IgG Y/N
25. anti-prothrombin IgM Y/N
26. anti-phosphatidylserine IgG Y/N
27. anti-phosphatidylserine IgM Y/N
28. lupus anticoagulant Y/N
29. direct Coombs test POS/NEG
30. anti-peroxidase Y/N
31. anti-thyroglobulin Y/N

Appendix 2: Study cohort tables

Table XVI: AIDs present in the study cohort

Autoimmune Disease	**No. patients**
Systemic lupus erythematosus	246
Sjögren's syndrome	109
Antiphospholipid syndrome	69
Autoimmune thyroid disease	23
Systemic sclerosis/CREST	15
Psoriasis	15
Primary biliary cirrhosis	15
Rheumatoid arthritis	8
Autoimmune hepatitis	8
Vitiligo	7
Spondiloarthropathies	4
Inflammatory intestinal disease (Crohn's disease and ulcerative colitis)	3
Pernicious anaemia	3
Cryoglobulinemia	3
Dermatomyositis/Polymyositis	3
Devic syndrome	2
Mixed connective tissue disease	2
Type 1 diabetes mellitus	2
ANCA+ vasculitis	2
Sarcoidosis	2
Kikuchi syndrome	1
Autoimmune cholangitis	1
Myasthenia gravis	1
Hyper IgG4	1
Non-ANCA+ vasculitis	1
Rheumatic polymyalgia	1
Eosinophilic pneumonia	1
Aquagenic pruritus	1

Appendix 3: SLE tables

Table XVII: SLE group – Genetic polymorphisms impact on clinical and immunologic variables

Genetic variables		Clinical and immunologic variables						
Class II HLA		**Organ involvement**		OR [95%CI]	p	**Antibodies**	OR [95%CI]	p
Susceptibility	DRB1*01	Mucocutaneous	chronic	3.02 [1.46-6.26]	0.002	(n.s.)		
	DRB1*03	(n.s.)				anti-La/ SSB	3.57 [1.74-7.32]	<0.001
	DRB1*04	NPSLE	global	2.19 [1.03-4.61]	0.038	(n.s.)		
			peripheral	7.10 [1.15-43.96]	0.045			
	DRB1*07	Musculoskeletal	muscles / tendons	2.22 [1.04-4.75]	0.036	(n.s.)		
	DRB1*08	(n.s.)				anti-dsDNA	7.71 [1.01-58.75]	0.021
						anti-Sm	4.89 [1.98-12.15]	<0.001
						anti-ribosomal P	4.31 [1.14-16.30]	0.043
	DRB1*09	(n.s.)				(n.s.)		
	DRB1*10	(n.s.)				aβ2GPI IgG	8.56 [1.82-40.18]	0.010
						aβ2GPI IgM	6.61 [1.25-35.01]	0.022
	DRB1*12	Gastrointestinal		10.21 [1.57-66.56]	0.040	(n.s.)		
	DRB1*15	Ophthalmologic		3.56 [1.09-11.56]	0.037	(n.s.)		
	DRB1*16	(n.s.)				anti-Ro/ SSA	3.32 [1.15-9.56]	0.020
						anti-peroxidase	6.00 [1.20-30.10]	0.046
Protection	DRB1*04	Mucocutaneous	chronic	0.31 [1.46-6.26]	0.015	RF	0.40 [0.17-0.91]	0.025
	DRB1*07	(n.s.)				(n.s.)		
	DRB1*08	Renal	global	0.28 [0.08-0.98]	0.035	(n.s.)		
			severe	0.22 [0.05-0.97]	0.030			
	DRB1*11	(n.s.)				low C3	0.44 [0.20-0.97]	0.039
						ANA	0.07 [0.01-0.83]	0.048
	DRB1*13	(n.s.)				anti-peroxidase	0.75 [0.67-0.84]	0.039
	DRB1*15	(n.s.)				low C3	0.49 [0.26-0.93]	0.027
	DRB1*16	(n.s.)				LAC	0.88 [0.81-0.95]	0.018
PTPN22	(n.s)							

Table XVIII: SLE group, monoautoimmunity subgroup – Genetic polymorphisms impact on clinical and immunologic variables

Genetic variables		**Mono SLE: Clinical and immunological variables**						
Class II HLA		**Organ involvement**		OR [95%CI]	p	**Antibodies**	OR [95%CI]	p
Susceptibility	DRB1*01	Mucocutaneous	chronic	3.81 [1.56-9.31]	0.002	(n.s.)		
	DRB1*03	(n.s.)				anti-La/SSB	3.28 [1.23-8,78]	0.014
	DRB1*07	(n.s.)				anti-dsDNA	2.84 [1.10-7.37]	0.027
	DRB1*08	(n.s.)				anti-Sm	10.69 [3.04-37.58]	<0.001
						anti-ribosomal P	7.03 [1.33-37.17]	0.037
	DRB1*12	(n.s.)				aβ2GPI IgG	11.33 [1.46-87.76]	0.043
	DRB1*15	Mucocutaneous	vasculitis	2.87 [1.18-7.00]	0.017	(n.s.)		
		Ophthalmologic		9.57 [1.77-51.86]	0.008	(n.s.)		
	DRB1*16	Renal	grade IV biopsy	1.33 [1.01-1.77]	0.007	(n.s.)		
Protection	DRB1*03	Musculoskeletal	global	0.28 [0.09-0.91]	0.034	(n.s.)		
			arthritis	0.45 [0.23-0.91]	0.024			
	DRB1*08	Raynaud's phenomenon		0.13 [0.02-1.01]	0.032	(n.s.)		
	DRB1*11	(n.s.)				anti-C1q	0.12 [0.02-0.96]	0.029
	DRB1*15	(n.s.)				low C3	0.43 [0.20-0.92]	0.028
PTPN22								
Susceptibility	PTPN22_T	Gastrointestinal		4.82 [1.12-20.75]	0.043	(n.s.)		

Table XIX: SLE group, MAS subgroup – Genetic polymorphisms impact on clinical and immunologic variables

Genetic variables		**MAS SLE: Clinical and immunological variables**						
Class II HLA		**Organ involvement**		OR [95%CI]	p	**Antibodies**	OR [95%CI]	p
Susceptibility	DRB1*03	(n.s.)				anti-La/SSB	3.50 [1.17-10.41]	0.021
	DRB1*04	NPSLE	peripheral	R=1.27 [0.97-1.67]	0.007	anti-ribosomal P	9.71 [1.48-63.81]	0.021
		Cardioresp	alveolitis/ pneumonitis	10.80 [1.74-67.09]	0.013	aβ2GPI IgG	5.46 [1.57-18.98]	0.015
			severe	5.10 [1.09-23.86]	0.048	(n.s.)		
	DRB1*07	(n.s.)				anti-U1RNP	6.85 [1.36-34.60]	0.010
	DRB1*13	(n.s.)				aβ2GPI IgM	3.79 [1.13-12.69]	0.025
	DRB1*15	(n.s.)				anti-nucleosome	6.00 [1.15-31.25]	0.021
	DRB1*16	Mucocut.	subacute	6.63 [1.38-31.93]	0.026	anti-Ro/ SSA	1.21 [1.06-1.37]	0.048
Protection	DRB1*03	(n.s.)				aCL IgG	0.25 [0.08-0.74]	0.010
	DRB1*04	Mucocut.	chronic	0.14 [0.02-1.14]	0.050	RF	0.17 [0.04-0.85]	0.019
	DRB1*13	(n.s.)				anti-peroxidase	0.68 [0.54-0.85]	0.042
	DRB1*15	NPSLE	central focal	0.67 [0.56-0.81]	0.004	(n.s.)		
PTPN22								
Protection	PTPN22_T	(n.s.)				aCL IgM	0.74 [0.62-0.88]	0.028

Table XX: SLE group - Antibody association with organ involvement

Antibodies		SLE group			
		Organ involvement		OR [95%CI]	p
Susceptibility	anti-dsDNA	Renal	global	3.36 [1.56-7.28]	0.001
			severe	3.77 [1.61-8.83]	0.001
		Cardiorespiratory	global	3.70 [1.40-9.82]	0.005
			serositis	2.67 [1.00-7.15]	0.044
		Hematologic	global	2.21 [1.10-4.46]	0.025
	anti-C1q	Renal	global	2.43 [1.15-5.12]	0.019
		Mucocutaneous	subacute	3.82 [1.31-11.18]	0.010
			chronic	3.10 [1.35-7.10]	0.006
	low C3	Renal	global	2.77 [1.43-5.36]	0.002
			severe	2.70 [1.34-5.43]	0.004
		Mucocutaneous	vasculitis	2.39 [1.01-5.67]	0.044
		Cardiorespiratory	global	4.42 [1.80-10.88]	0.001
			serositis	5.19 [1.78-15.13]	0.001
		Hematologic	global	2.26 [1.16-4.41]	0.015
			haemolytic anaemia	6.56 [1.52-28.38]	0.004
			thrombocytopenia	1.89 [1.01-3.54]	0.045
			severe	6.61 [1.53-28.59]	0.004
		Gastrointestinal		6.32 [0.82-49.03]	0.045
	low C4	Renal	global	2.31 [1.27-4.18]	0.005
			severe	2.26 [1.21-4-25]	0.010
		Cardiorespiratory	global	4.38 [1.96-9.79]	<0.001
			serositis	3.65 [1.55-8.59]	0.002
			alveolitis/ pneumonitis	7.32 [0.94-57.29]	0.035
			severe	10.34 [1.35-79.24]	0006
		Hematologic	haemolytic anaemia	4.05 [1.36-12.07]	0.007
			severe	4.09 [1.37-12.16]	0.007
	anti-Ro/SSA	NPSLE	global	1.90 [1.02-3.53]	0.042
		Mucocutaneous	subacute	3.71 [1.51-9.11]	0.003
		Raynaud's phenomenon		1.93 [1.15-3.23]	0.012
	anti-La/SSB	Musculoskeletal	muscle/tendon	0.33 [0.16-0.70]	0.008
		Hematologic	haemolytic anaemia	2.94 [1.21-7.12]	0.024
			severe	2.92 [1.21-7.08]	0.025
	anti-Sm	Renal	grade IV biopsy	4.15 [1.47-11.78]	0.006
		Mucocutaneous	acute	3.28 [1.11-9.64]	0.024
			chronic	1.92 [1.00-3.67]	0.048
		Cardiorespiratory	severe	3.53 [1.28-9.74]	0.028
	anti-U1RNP	Renal	global	1.79 [1.02-3.12]	0.041
		Mucocutaneous	vasculitis	2.18 [1.11-4.30]	0.022
		Raynaud's phenomenon		2.57 [1.48-4.44]	0.001
	anti-ribosomal P	Renal	global	2.90 [1.12-7.51]	0.023
		Cardiorespiratory	endocarditis	13.79 [1.19-159-51]	0.050
			alveolar haemorrhage	R=1.11	0.018
			severe	4.04 [1.21-13.52]	0.032
	anti-nucleosome	Cardiorespiratory	severe	3.60 [0.99-13.10]	0.040
		Hematologic	global	2.42 [1.12-5.22]	0.022
	anti-CCP	Hematologic	thrombocytopenia	R=1.20	0.019
	RF	Musculoskeletal	arthritis	1.91 [1.06-3.42]	0.030
			muscle/tendon	2.19 [1.02-4.69]	0.040
			global	3.24 [1.65-6.36]	<0.001

	aPL	NPSLE	peripheral	R=1.05	0.030
			central focal	2.57 [1.24-5.32]	0.009
			central diffuse	4.30 [1.19-15.49]	0.016
		Cardiorespiratory	alveolitis/ pneumonitis	5.40 [1.17-24.93]	0.016
			severe	3.43 [1.09-10.78]	0.026
		Gastrointestinal		3.93 [1.08-14.30]	0.027
	aCL IgG	NPSLE	global	2.25 [1.16-4.38]	0.016
			central focal	2.53 [1.23-5.18]	0.010
		Hematologic	global	2.76 [1.03-7.39]	0.036
			haemolytic anaemia	2.24 [1.00-5.08]	0.049
	aCL IgM	NPSLE	global	2.64 [1.37-5.10]	0.003
			central focal	2.08 [1.01-4.27]	0.043
			central diffuse	3.46 [1.24-9.69]	0.029
		Cardiorespiratory	alveolitis/ pneumonitis	4.00 [1.29-12.44]	0.018
		Hematologic	haemolytic anaemia	2.52 [1.12-5.65]	0.022
			severe	2.50 [1.12-5.61]	0.023
		Raynaud's associated ulcers		2.08 [1.03-4.20]	0.038
	aβ2GPI IgG	Renal	global	2.03 [1.00-4.12]	0.048
		NPSLE	global	2.19 [1.03-4.68]	0.039
			central focal	2.43 [1.09-5.42]	0.027
		Cardiorespiratory	alveolitis/ pneumonitis	5.39 [1.70-17.10]	0.007
			severe	4.46 [1.58-12.55]	0.008
	aβ2GPI IgM	NPSLE	global	2.10 [1.11-3.98]	0.021
			central diffuse	3.49 [1.25-9.80]	0.020
	LAC	Hematologic	thrombocytopenia	2.76 [1.32-5.76]	0.006
Protection	low C4	Musculoskeletal	global	0.19 [0.06-0.65]	0.004
			arthralgia	0.24 [0.08-0.73]	0.007
	anti-La/SSB	Mucocutaneous	ulcers	0.31 [0.12-0.84]	0.016
	anti-Sm	NPSLE	global	0.37 [0.15-0.93]	0.029
	anti-Ro52	Mucocutaneous	chronic	0.02 [0.00-0.45]	0.016
		Musculoskeletal	muscle & tendon	0.02 [0.00-0.50]	0.016
	anti-centromere	Mucocutaneous	acute	0.19 [0.04-0.86]	0.040
	aβ2GPI IgG	Musculoskeletal	global	0.30 [0.12-0.74]	0.019
			arthralgia	0.32 [0.13-0.78]	0.021
	aβ2GPI IgM	Mucocutaneous	ulcers	0.48 [0.24-0.95]	0.034
		Ophthalmologic		0.15 [0.02-1.17]	0.045
	LAC	Mucocutaneous	global	0.32 [0.12-0.90]	0.025
			acute	0.23 [0.09-0.56]	0.001
		Musculoskeletal	global	0.19 [0.07-053]	0.001
			arthralgia	0.19 [0.07-0.53]	0.001
			arthritis	0.33 [0.16-0.70]	0.003

(Cardiorespiratory severe=all involvement except serositis; Haematologic severe=haemolytic anemia or haemophagocytic syndrome (vs. other involvement/no involvement); Renal severe=biopsy grade III, IV, V or VI or renal failure or nephritic or nephrotic syndrome)

Table XXI: SLE group, mono & MAS subgroups – Antibody association with organ involvement

Antibodies		Mono SLE				MAS SLE			
		Organ involvement		OR [95%CI]	p	Organ involvement		OR [95%CI]	p
Susceptibility	anti-dsDNA	Renal	global	4.50 [1.64-12.36]	0.002	Cardiorespiratory	global	11.30 [1.41-90.34]	0.006
			severe	4.60 [1.52-13.90]	0.004				
		Musculoskeletal	arthritis	2.61 [1.22-5.56]	0.011				
		Hematologic	global	2.83 [1.26-6.37]	0.010				
	anti-C1q	Renal	global	4.81 [1.83-12.65]	0.001	Mucocutaneous	chronic	5.67 [1.22-26.33]	0.020
			severe	3.07 [1.16-8.11]	0.021				
		Mucocutaneous	subacute	5.09 [1.17-22.16]	0.028				
	low C3	Renal	global	2.59 [1.20-5.59]	0.013	Cardiorespiratory	global	13.20 [1.66-105.21]	0.003
			severe	2.58 [1.13-5.89]	0.021		serositis	8.20 [1.02-66.10]	0.003
		Cardiorespiratory	global	2.80 [1.01-7.76]	0.042	Hematologic	haemolytic anaemia	R=1.24	0.029
			serositis	4.18 [1.20-14.63]	0.017				
		Hematologic	global	2.30 [1.08-4.90]	0.028				
	low C4	Renal	global	2.12 [1.06-4.25]	0.032	Renal	severe	3.89 [1.02-14.75]	0.037
		Cardiorespiratory	global	3.22 [1.24-8.36]	0.013	Cardiorespiratory	global	7.88 [1.68-36.88]	0.003
			serositis	3.16 [1.13-8.85]	0.023		serositis	4.74 [1.00-22.57]	0.036
		Hematologic	global	2.22 [1.06-4.65]	0.032	Hematologic	haemolytic anaemia	R=1.26	0.013
							severe	1.26 [1.01-1.45]	0.013
	anti-Ro/ SSA	Renal	global	1.96 [1.02-3.79]	0.044	Mucocutaneous	subacute	8.00 [1.00-65.10]	0.029
		Mucocutaneous	chronic	2.49 [1.21-5.15]	0.012				
		Cardiorespiratory	severe	3.99 [1.0-16.06]	0.048				
		Raynaud's phenomenon		1.91 [1.00-3.67]	0.049				
	anti-La/SSB	Cardiorespiratory	CAD	1.13 [0.95-1.35]	0.011	Musculoskeletal	muscle/tendon	4.00 [1.31-12.26]	0.017
	anti-Sm	Renal	severe	4.23 [1.22-14.62]	0.049	Raynaud's phenomenon		3.68 [0.96-14.08]	0.047
		Mucocutaneous	acute	4.59 [1.04-20.37]	0.030				
			chronic	2.40 [1.08-5.33]	0.030				
	anti-U1RNP	Renal	global	2.08 [1.04-4.18]	0.038	(n.s.)			
		Mucocutaneous	vasculitis	2.42 [1.05-5.61]	0.036				
		Raynaud's phenomenon		2.46 [1.23-4.89]	0.010				
	anti-ribosomal P	Renal	global	4.22 [1.22-14.57]	0.016	Cardiorespiratory	global	7.08 [1.21-41.46]	0.028
							endocarditis	R=1.40	0.015

							severe	9.50 [1.42-63-72]	0.033
	anti-nucleosome	Hematologic	global	2.60 [1.06-4.65]	0.028	(n.s.)			
		Raynaud's phenomenon		2.10 [1.00-4.41]	0.048				
	anti-CCP	(n.s.)				Hematologic	thrombocytopenia	1.75 [0.92-3.32]	0.020
	anti-centromere	(n.s.)				Renal	global	10.40[1.03-104-72]	0.039
	RF	Renal	severe	4.23 [1.15-5.38]	0.019	(n.s.)			
	aPL	Renal	severe	3.00 [1.0-9.1]	0.049	Mucocutaneous	chronic	4.97 [1.05-23.55]	0.029
		NPSLE	global	3.02 [1.24-7.35]	0.012				
			central focal	2.64 [1.03-6.78]	0.039				
	aCL IgG	(n.s.)				Cardiorespiratory	alveolitis/ pneumonitis	9.60 [1.06-86.71]	0.027
						Hematologic	global	R=1.71	0.040
	aCL IgM	NPSLE	global	2.70 [1.13-6.93]	0.023	Cardiorespiratory	alveolitis/ pneumonitis	6.11 [1.03-36.20]	0.048
			central focal	2.64 [1.03-6.78]	0.039				
	aβ2GPI IgG	(n.s.)				Cardiorespiratory	global	2.82 [1.00-8.00]	0.047
							alveolitis/ pneumonitis	16.47 [1.80-150.83]	0.006
							severe	10.07 [1.83-55.57]	0.006
	aβ2GPI IgM	(n.s.)				Raynaud's phenomenon		3.39 [1.17-9.77]	0.021
						Raynaud's associated ulcers		3.31 [1.12-9.88]	0.028
	LAC	Mucocutaneous	vasculitis	3.78 [1.31-10.93]	0.017	Renal	global	5.00 [1.23-20.41]	0.037
Protection	anti-dsDNA	Mucocutaneous	chronic	0.43 [0.20-0.96]	0.036	(n.s.)			
	anti-C1q	NPSLE	global	R= 0.63 [0.53-0.75]	0.008	(n.s.)			
	low C4	Musculoskeletal	global	0.23 [0.07-0.78]	0.019	(n.s.)			
			arthralgia	0.09 [0.01-0.72]	0.006				
	anti-La/SSB	(n.s.)				Mucocutaneous	ulcers	0.18 [0.04-0.83]	0.017
	anti-Sm	NPSLE	global	0.13 [0.02-0.99]	0.022	(n.s.)			
	anti-U1RNP	NPSLE	central focal	0.23 [0.05-1.03]	0.039	(n.s.)			
	anti-centromere	(n.s.)				Mucocutaneous	acute	0.08 [0.01-0.66]	0.028
	RF	(n.s.)				Gastrointestinal		0.51 [0.41-0.65]	0.030

	aPL	Musculoskeletal	muscle/ tendon	0.29 [0.10-0.91]	0.027	(n.s.)			
		Raynaud's phenomenon		0.45 [0.23-0.89]	0.020				
	aβ2GPI IgM	Mucocutaneous	ulcers	0.37 [0.14-0.95]	0.034	Ophthalmologic		R=0.84	0.021
		Musculoskeletal	muscle / tendon	0.13 [0.02-1.03]	0.025				
	LAC	Musculoskeletal	global	0.23 [0.07-0.78]	0.019	Mucocutaneous	global	R=0.778	0.017
			arthralgia	0.23 [0.07-0.78]	0.019		acute	0.06 [0.01-0.51]	0.003
			arthritis	0.39 [0.15-0.99]	0.045	Musculoskeletal	global	0.09 [0.01-0.88]	0.025
							arthralgia	0.09 [0.01-0.87]	0.025
							arthritis	0.27 [0.08-0.97]	0.039

(aβ2GPI=anti- β2glycoproteinI antibodies; aPL=antiphospholipid antibodies; aCL=anticardiolipin antibodies; CAD=coronary artery disease; LAC=lupus anticoagulant; NPSLE=Neuropsychiatric SLE; RF=rheumatoid factor; renal severe=biopsy grade III, IV, V or VI or renal failure or nephritic or nephrotic syndrome; cardiorespiratory severe=all involvement except serositis; haematologic severe=haemolytic anaemia or haemophagocytic syndrome (vs. other involvement/no involvement)

Appendix 4: SjS tables

Table XXII: SjS group – Genetic polymorphisms impact on clinical and immunologic variables

Genetic variables		Clinical and immunological variables					
Class II LA		**Organ involvement**	OR [95%CI]	p	**Atbs**	OR [95%CI]	p
Susceptibility	DRB1*01	(n.s.)			anti-C1q	1.67 [1.01-2.77]	0.020
	DRB1*03	(n.s.)			anti-La/SSB	2.95 [1.31-6.68]	0.009
	DRB1*04	(n.s.)			LAC	11.50 [1.84-72.07]	0.008
	DRB1*07	(n.s.)			anti-U1RNP	5.13 [1.72-15.29]	0.002
					aCL IgM	4.46 [1.40-14.16]	0.014
	DRB1*10	(n.s.)			aCL IgM	15.80 [1.54-162.31]	0.019
	DRB1*13	Other - Raynaud associated ulcers	3.76 [1.14-12.44]	0.034	low C3	3.13 [1.07-9.16]	0.032
					low C4	5.93 [1.91-18.42]	0.001
					aPL	4.30 [1.38-13.43]	0.008
					aCL IgM	4.46 [1.40-14.16]	0.014
					aβ2GPI IgG	4.41 [1.20-16.16]	0.032
					aβ2GPI IgM	6.29 [2.06-19.20]	0.001
Protection	DRB1*01	Bad prognosis - low complement	0.27 [0.07-1.04]	0.046	anti-Ro/ SSA	0.17 [0.05-0.57]	0.006
	DRB1*03	(n.s.)			aCL IgG	0.28 [0.08-0.95]	0.033
	DRB1*04	(n.s.)			anti-La/SSB	0.20 [0.05-0.75]	0.011
	DRB1*07	(n.s.)			low C4	0.27 [0.08-1.00]	0.039
					LAC	0.68 [0.52-0.89]	0.036
	DRB1*13	Sicca syndrome	0.09 [0.02-0.53]	0.009	anti-peroxidase	0.69 [0.56-0.85]	0.047
PTPN22							
Susceptibility	PTPN22_T	Pulmonary involvement	5.92 [1.36-25.78]	0.027	(n.s.)		

Table XXIII: SjS group, monoautoimmunity subgroup – Genetic polymorphisms impact on clinical and immunologic variables

Genetic variables		**Mono SjS - Clinical and immunological variables**						
Class II HLA		**Organ involvement**		OR [95%CI]	p	**Antibodies**	OR [95%CI]	p
Susceptibility	DRB1*01	(n.s.)				anti-peroxidase	21.00 [1.40-314.04]	0.032
	DRB1*07	(n.s.)				aCL IgM	R=1.40	0.026
	DRB1*13	Bad prognosis	Adenopathies	7.25 [1.12-47.00]	0.042	(n.s.)		
	DRB1*15	Raynaud's associated ulcers		R=1.33	0.018	(n.s.)		
Protection	DRB1*01	(n.s.)				anti-Ro/SSA	0.07 [0.01-0.46]	0.008
	DRB1*03	(n.s.)				low C3	0.21 [0.05-0.85]	0.037
	DRB1*04	(n.s.)				anti-La/SSB	0.75 [0.58-0.97]	0.016
	DRB1*15	(n.s.)				aPL	0.53 [0.39-0,73]	0.031
PTPN22								
Susceptibility	PTPN22_T	(n.s.)				anti-peroxidase	2.00 [0.75-5.33]	0.029
Protection	PTPN22_T	(n.s.)				anti-Ro/SSA	0.05 [0.00-0.54]	0.014

Table XXIV: SjS group, MAS subgroup – Genetic polymorphisms impact on clinical and immunologic variables

Genetic variables		MAS SjS - Clinical and immunological variables						
Class II HLA		**Organ involvement**		OR [95%CI]	p	**Atbs**	OR [95%CI]	p
Susceptibility	DRB1*01	(n.s.)				anti-C1q	1.67 [1.01-2.77]	0.029
						anti-nucleosome	1.33 [1.01-1.77]	0.039
	DRB1*03	Bad prognosis	Adenopathy	4.24 [1.00-18.22]	0.042	anti-La/SSB	4.68 [1.52-14.40]	0.006
	DRB1*04	(n.s.)				LAC	20.40 [1.97-211.79]	0.006
	DRB1*07	(n.s.)				anti-U1RNP	14.50 [1.69-124.24]	0.004
	DRB1*10	(n.s.)				aβ2GpI IgG	1.22 [0.93-1.62]	0.034
	DRB1*13	Pulmonary involvement		6.83 [1.28-36.58]	0.032	low C4	5.44 [1.29-23.00]	0.014
						aPL	10.52 [1.25-88.44]	0.018
		Raynaud's associated ulcers		7.60 [1.75-32.93]	0.008	aCL IgM	5.60 [1.44-21.83]	0.025
						aβ2GPI IgG	4.64 [1.11-19.48]	0.042
						aβ2GPI IgM	7.39 [1.72-31.70]	0.006
	DRB1*15	(n.s.)				anti-U1RNP	4.47 [1.21-16.44]	0.019
Protection	DRB1*01	Bad prognosis	global	0.12 [0.02-0.62]	0.017	(n.s.)		
			hyper Ig	0.14 [0.03-0.72]	0.021			
	DRB1*03	(n.s.)				aCL IgG	0.22 [0.05-0.89]	0.025
	DRB1*04	(n.s.)				RF	0.17 [0.03-0.87]	0.022
	DRB1*08	(n.s.)				aPL	0.09 [0.01-0.77]	0.014
						aβ2GPI IgM	0.80 [0.68-0.94]	0.036
	DRB1*13	Sicca syndrome		0.10 [0.02-0.61]	0.016	(n.s.)		
PTPN22								
Susceptibility	PTPN22_T	(n.s.)				low C3	1.32 [1.06-1.64]	0.022
						anti-CCP	32.00 [1.30-737.46]	0.046

Table XXV: SjS group – Antibody association with organ involvement

Antibodies		SSj group			
		Organ involvement		p	OR [95%CI]
Susceptibility	anti-dsDNA	Bad prognosis criteria	low complement	3.68 [1.60-8.47]	0.002
		Raynaud's phenomenon		2.55 [1.13-5.75]	0.022
	low C3	Bad prognosis criteria	global	4.67 [1.26-17.28]	0.013
			low complement	30.86 [10.54-90.32]	<0.001
	low C4	Bad prognosis criteria	low complement	33.75 [10.77-105.71]	<0.001
	anti-Ro/ SSA	Bad prognosis criteria	hyper Ig	4.42 [1.44-13.55]	0.006
	anti-La/ SSB	Bad prognosis criteria	global	3.74 [1.14-12.26]	0.022
			hyper Ig	3.71 [1.50-9.20]	0.004
	anti-Sm	Raynaud's phenomenon		3.66 [1.10-12.20]	0.027
		Pulmonary involvement		4.79 [1.43-16.06]	0.015
	anti-U1RNP	Bad prognosis criteria	hyper Ig	5.53 [1.75-17.50]	0.002
		Raynaud's phenomenon		3.13 [1.35-7.23]	0.007
	anti-centromere	Bad prognosis criteria	cryoglobulines	R=3.75	0.004
	RF	Bad prognosis criteria	parotid enlargement	3.86 [1.03-14.42]	0.035
			hyper Ig	6.46 [2.53-16.52]	<0.001
	aPL	Bad prognosis criteria	global	4.26 [1.15-15.76]	0.021
			low complement	3.26 [1.43-7.41]	0.004
			hyper Ig	3.60 [1.37-9.46]	0.007
	aCL IgG	Bad prognosis criteria	low complement	0.050	2.92 [0.97-8.77]
	aCL IgM	Bad prognosis criteria	hyper Ig	R=1.70	0.001
		Neurologic involvement		5.64 [1.44-22.07]	0.018
	aβ2GPI IgG	Bad prognosis criteria	low complement	6.15 [1.58-23.95]	0.004
		Raynaud's associated ulcers		4.70 [1.28-17.23]	0.026
	aβ2GPI IgM	Bad prognosis criteria	low complement	2.89 [1.15-7.29]	0.022
			hyper Ig	7.93 [1.74-36.24]	0.003
		Raynaud's associated ulcers		4.57 [1.54-13.59]	0.011
Protection	anti-dsDNA	Bad prognosis criteria	parotid enlargement	0.09 [0.01-0.67]	0.004
	aPL	Sicca syndrome		0.10 [0.01-0.86]	0.017
	aCL IgG	Sicca syndrome		0.10 [0.02-0.52]	0.010
	aβ2GPI IgG	Sicca syndrome		0.15 [0.03-0.77]	0.038

Table XXVI: SjS group, mono & MAS subgroups – Antibody association with organ involvement

Antibodies		Mono SSj			MAS SSj		
		Organ involvement	OR [95%CI]	p	Organ involvement	OR [95%CI]	p
Susceptibility	anti-dsDNA	(n.s.)			Bad prognosis - low complement	3.72 [1.24-11.17]	0.017
	low C3	Bad prognosis			Bad prognosis - low complement	134.40 [14.67-1231.51]	<0.001
		- global	R=1.40	0.046			
		- low complement	10.00 [2.62-44.20]	0.002			
	low C4	Bad prognosis			Bad prognosis - low complement	22.75 [5.36-96.61]	<0.001
		- global	R=1.42	0.043			
		- adenopathies	5.64 [1.39-22.88]	0.024			
		- low complement	58.67 [8.64-398.51]	<0.001			
	anti-Ro/SSA	Bad prognosis - hyper Ig	6.00 [1.06-33.96]	0.047	(n.s.)		
	anti-La/SSB	Bad prognosis - hyper Ig	4.57 [1.31-15.98]	0.014	(n.s.)		
	anti-Sm	Pulmonary involvement	10.80 [1.43-81.33]	0.031	(n.s.)		
	anti-U1RNP	Bad prognosis - hyper Ig	R=2.00	0.007	Raynaud's phenomenon	3.24 [1.04-10.10]	0.039
	anti-centrom	(n.s.)			Bad prognosis - cryoglobulines	R=2.83	0.035
	RF	Bad prognosis - hyper Ig	15.00 [2.70-83.45]	0.001	Bad prognosis - hyper Ig	9.86 [1.92-50.70]	0.002
	aPL	Bad prognosis - low complement	6.25 [1.23-31.90]	0.031	Bad prognosis - hyper Ig	5.63 [1.47-21.57]	0.008
	aCL IgM	Neurologic involvement	25.33 [1.75-366.83]	0.030	Bad prognosis - hyper Ig	R=1.58	0.005
	aβ2GPI IgG	(n.s.)			Bad prognosis - low complement	7.11 [1.40-36.18]	0.010
	aβ2GPI IgM	(n.s.)			Bad prognosis - hyper Ig	12.35 [1.47-103-79]	0.006
					Raynaud's associated ulcers	5.71 [1.49-21.84]	0.010
	anti-thyroglobulin	(n.s.)			Bad prognosis - parotid enlargement	R=1.43	0.020
Protection	anti-U1RNP	(n.s.)			Bad prognosis - adenopathies	0.20 [0.04-1.05]	0.042
	aPL	(n.s.)			Sicca syndrome	R=0.83	0.036
	aCL IgG	(n.s.)			Sicca syndrome	0.13 [0.02-0.80]	0.030

Appendix 5: APS tables

Table XXVII: APS group – Genetic polymorphisms impact on clinical and immunologic variables

Genetic variables		Clinical and immunological variables					
Class II HLA		**Organ involvement**	OR [95%CI]	p	**Antibodies**	OR [95%CI]	p
Susceptibility	DRB1*01	Other manifestations			(n.s.)		
		- memory problems	8.04 [1.73-37.38]	0.011			
		- avascular hip necrosis	1.29 [0.91-1.82]	0.020			
	DRB1*03	(n.s.)			anti-La/ SSB	5.33 [1.24-22.88]	0.037
					RF	3.89 [1.15-13.14]	0.024
	DRB1*04	(n.s.)			aβ2GPI IgG	6.28 [1.74-22.65]	0.003
	DRB1*08	(n.s.)			anti-Sm	14.70 [1.97-109.92]	0.015
					anti-nucleosome	R=2.40	0.042
	DRB1*13	Non-thrombotic manifestations - thrombocytopenia	5.67 [1.32-24.25]	0.018	LAC	9.92 [1.08-91.47]	0.042
	DRB1*14	Thrombotic manifestations - obstetric	1.18 [1.00-1.39]	0.031	(n.s.)		
	DRB1*15	(n.s.)			anti-U1RNP	5.20 [1.29-20.89]	0.023
					anti-nucleosome	9.23 [1.00-85.78]	0.040
	DRB1*16	(n.s.)			anti-Ro/SSA	R=3.31	0.002
					RF	6.33 [1.03-38.98]	0.049
Protection	DRB1*03	(n.s.)			aCL IgG	0.30 [0.10-0.89]	0.028
	DRB1*04	(n.s.)			RF	0.12 [0.02-1.04]	0.043
	DRB1*07	(n.s.)			anti-dsDNA	0.23 [0.06-0.96]	0.034
					low C4	0.15 [0.04-0.62]	0.008
	DRB1*13	(n.s.)			anti-U1RNP	0.69 [0.57-0.83]	0.050
	DRB1*15	(n.s.)			aβ2GPI IgG	0.21 [0.04-1.05]	0.042
	DRB1*16	(n.s.)			aβ2GPI IgG	0.50 [0.38-0.64]	0.027
PTPN22		**Organ involvement**	OR [95%CI]	p	**Antibodies**	OR [95%CI]	p
Susceptibility	PTPN22_T	Other manifestations			(n.s.)		
		- memory problems	5.28 [1.22-22.87]	0.032			
		- seizures	16.13 [1.49-175.22]	0.022			
Protection	PTPN22_T	(n.s.)			aCL IgM	0.20 [0.04-1.05]	0.042

Table XXVIII: APS group, monoautoimmunity subgroup – Genetic polymorphisms impact on clinical and immunologic variables

Genetic variables		**Mono APS - Clinical and immunological variables**					
Class II HLA		**Organ damage**	OR [95%CI]	p	**Antibodies**	OR [95%CI]	p
Susceptibility	DRB1*07	(n.s.)			aβ2GP I IgG	R=2.5	0.038
	DRB1*13	(n.s.)			LAC	R=8.00	0.024
	DRB1*15	Other manifestations - visual disturbances	R=9.01	0.032	(n.s.)		
Protection	-	(n.s.)			(n.s.)		
PTPN22		(n.s.)			(n.s.)		

Table XXIX: APS group, MAS subgroup – Genetic polymorphisms impact on clinical and immunologic variables

Genetic variables		**MAS APS - Clinical and immunological variables**					
Class II HLA		**Organ damage**	OR [95%CI]	p	**Antibs.**	OR [95%CI]	p
Susceptibility	DRB1*01	Other manifestations			(n.s.)		
		- memory problems	7.73 [1.31-45.51]	0.031			
		- avascular hip necrosis	1.40 [0.88-2.24]	0.026			
	DRB1*03	(n.s.)			anti-La/ SSB	5.07 [1.10-23.45]	0.040
	DRB1*04	(n.s.)			aβ2GPI IgG	7.70 [1.35-43.88]	0.021
	DRB1*08	(n.s.)			anti-Sm	9.00 [1.19-68.13]	0.046
	DRB1*11	(n.s.)			RF	10.00 [1.00-100.82]	0.043
	DRB1*13	Non-thrombotic manifestations - thrombocytopenia	14.40 [1.53-135.52]	0.009	(n.s.)		
		Other manifestations - visual disturbances	8.13 [1.32-50.21]	0.024			
	DRB1*16	(n.s.)			anti-Ro/SSA	R=2.12	0.024
	DRB1*07	(n.s.)			anti-dsDNA	0.13 [0.02-0.76]	0.031
					low C3	0.16 [0.03-0.91]	0.047
					low C4	0.16 [0.03-0.91]	0.047
	DRB1*08	(n.s.)			anti-Ro/ SSA	0.37 [0.24-0.57]	0.013
	DRB1*11	(n.s.)			low C3	0.05 [0.005-0.53]	0.009
	DRB1*13	(n.s.)			anti-U1RNP	0.55 [0.40-0.75]	0.033
	DRB1*16	(n.s.)			aβ2GPI IgG	0.51 [0.37-0.71]	0.033
PTPN22							
Susceptibility	PTPN22_T	Other manifestations - memory problems	7.33 [1.17-46.05]	0.042	(n.s.)		
Protection	PTPN22_T	(n.s.)			LAC	0.31 [0.15-0.65]	0.026

Table XXX: APS group – Antibody association with organ involvement

Antibodies		All APS		
		Organ damage	OR [95%CI]	p
Susceptibility	anti-dsDNA	Non-thrombotic manifestations		
		- global	5.40 [1.33-21.89]	0.012
		- Raynaud's phenomenon	4.20 [1.49-11.87]	0.006
	anti-Ro/SSA	Non-thrombotic manifestations - Raynaud's phenomenon	3.12 [1.10-8.86]	0.030
	anti-Sm	Other manifestations - avascular hip necrosis	1.29 [1.00-1.82]	0.016
	aCL IgG	Non-thrombotic manifestations - thrombocytopenia	3.53 [1.26-9.87]	0.014
	aβ2GPI IgM	Thrombotic manifestations - obstetric	3.17 [1.15-8.73]	0.024
	anti-prothrombin IgG	Other manifestations - global	R=1.82	0.027
	anti-prothrombin IgM	Other manifestations - psychiatric	33.33 [2.83-392.60]	0.003
	anti-phosphatidilserine IgG	Non-thrombotic manifestations - thrombocytopenia	6.93 [1.29-37.22]	0.027
Protection	low C3	Other manifestations - sleep disturbances	0.25 [0.09-0.74]	0.010
	aCL IgM	Non-thrombotic manifestations		
		- global	0.20 [0.05-0.81]	0.016
		- migraine	0.36 [0.13-0.95]	0.038
	anti-peroxidase	Other manifestations - psychiatric	0.07 [0.01-0.64]	0.010

Table XXXI: APS group, mono & MAS subgroups – Antibody association with organ involvement

Antibodies		Mono APS			MAS APS		
		Organ damage	OR [95%CI]	p	**Organ damage**	OR [95%CI]	p
Susceptibility	anti-Sm	(n.s.)			Other manifestations - avascular hip necrosis	1.29 [0.91-1.82]	0.036
	aCL IgG	(n.s.)			Non-thrombotic manifestations		
					- global	R=1.29	0.019
					- thrombocytopenia	5.67 [1.55-20.69]	0.007
					Other manifestations - balance/vertigo	3.81 [1.00-14.67]	0.046
	aCL IgM	(n.s.)			Non-thrombotic manifestations - global	1.36 [1.04-1.78]	0.008
					Other manifestations - balance/vertigo	4.20 [1.13-15.59]	0.027
	aβ2GPI IgM	(n.s.)			Thrombotic manifestations - obstetric	4.18 [1.13-15.42]	0.027
					Non-thrombotic manifestations - Raynaud's phenomenon	5.29 [1.44-19.45]	0.010
	anti-prothrombin IgG	(n.s.)			Other manifestations		
					- visual disturbances	16.00 [1.27-200.92]	0.031
					- balance/vertigo	13.75 [1.48-127.47]	0.022
	anti-prothrombin IgM	(n.s.)			Other manifestations - psychiatric	30.00 [2.19-410.99]	0.007
	anti-phosphatidilserine IgG	(n.s.)			Other manifestations - visual disturbances	2.25 [1.08-4.67]	0.008
	LAC	(n.s.)			Other manifestations - visual disturbances	1.50 [1.05-2.15]	0.044
Protection	low C3	Other manifestations - psychiatric	0.57 [0.36-0.90]	0.048	(n.s.)		
	anti-U1RNP	(n.s.)			Other manifestations - psychiatric	0.19 [0.04-0.79]	0.017
	aCL IgG	Other manifestations - visual disturbances	0.60 [0.36-0.99]	0.024	(n.s.)		
	aβ2GPI IgM	Other manifestations - global	0.09 [0.009-0.97]	0.040	(n.s.)		
	anti-peroxidase	(n.s.)			Other manifestations - psychiatric	0.10 [0.01-0.93]	0.038

Resumo em Português

Introdução

As doenças autoimunes (DAIs) são entidades complexas e crónicas, que se devem à perda de tolerância imunológica a antigénios do próprio.[1-12] A sua prevalência mundial estimada é de 3-9.4%.[14-17] e como grupo, representam um ónus sobre os recursos médicos e sociais, com impacto na qualidade de vida.[3-5, 18] A capacidade de antever estas doenças numa fase pré-sintomática ou de prever a sua evolução representaria um importante passo no desenvolvimento de estratégias de prevenção.

Monoautoimunidade e poliautoimunidade

A maioria das DAI ocorre como uma doença isolada num determinado indivíduo (monoautoimunidade). No entanto, a existência de subfenótipos clínicos comuns a várias DAIs sugere uma partilha de mecanismos patofisiológicos, com factores de risco genéticos e ambientais idênticos – ou seja, uma possível origem comum: tautologia autoimune.[4-5, 8-9, 11-12, 19-23] Esta hipótese é corroborada por três níveis de evidência: 1) observações clínicas que indicam uma possível transformação de uma doença noutra ao longo do tempo ou a coexistência de mais de uma DAI num doente (poliautoimunidade) ou família (autoimunidade familiar); 2) mecanismos patofisiológicos já identificados e partilhados por várias DAIs e 3) estudos que sugerem factores genéticos comuns.[8-9, 19, 21] O *Multiple Autoimmune Syndrome* (MAS), que representa a coexistência de três ou mais DAIs num só indivíduo,[4-5, 7, 9] demonstra que a poliautoimunidade é mais do que mera coincidência.[5, 9, 22, 24-26]

Os *chaperones* da autoimunidade[3, 5, 19-20, 27] são doenças que, quando presentes, assinalam uma probabilidade aumentada de desenvolvimento de outras DAIs.[27-28] São eles: doença tiroideia autoimune (DTAI), lúpus eritematoso sistémico (SLE), síndrome de Sjögren's (SjS) e síndrome antifosfolípido (APS).

Doenças monogénicas vs. poligénicas

A maioria das DAI é poligénica – como tal, não é possível atribuir causalidade genética directa. A autoimunidade surge do pleiotropismo e epistase genéticos, modificados por factores ambientais. Como tal, a identificação dos polimorfismos genéticos envolvidos e suas possíveis interacções é um passo fundamental para a compreensão do fenómeno autoimune.

Genética e poliautoimunidade

O mecanismo patológico responsável pela coexistência de DAIs não é ainda compreendido. O facto de vários fenótipos autoimunes partilharem genes de susceptibilidade sugere um fundo genético comum. Apesar de não ser claro o mecanismo pelo qual os polimorfismos do HLA classe II conferem susceptibilidade,[10] a sua associação com diferentes DAIs está já bem documentada[2, 6, 12, 17, 31-37], reforçando o papel do HLA como factor de risco genético major.[36] A emergência dos *genome-wide association studies* (GWAS) levou à identificação de outros genes de susceptibilidade[17, 20, 35, 38], nomeadamente *single nucleotide polymorphisms* (SNPs).[14-15, 17, 35, 39-42] Destes, o mais estudado é provavelmente o PTPN22,[17, 43] que codifica uma proteína com importante papel supressor no sistema imunitário.[43] O polimorfismo Lyp620W (ou rs2476601) tem sido associado a um risco aumentado de DAI[15, 39, 42, 44-48] e possivelmente de MAS.[1]

Dada a comprovada associação entre HLA e PTPN22 e as doenças autoimunes, é possível que estes possam ser usados como marcadores de autoimunidade.

O objectivo do presente estudo é caracterizar e comparar o doente monoautoimune e o doente MAS, construindo uma perspectiva global dos mesmos. Pretende-se compreender se a poliautoimunidade influencia o curso da doença, particularmente em termos de severidade. Paralelamente, pretende-se perceber se a coocorrência dos três *chaperones* sistémicos de autoimunidade (SLE, SjS e APS) se traduz numa evolução diferente em termos de gravidade, em comparação com os outros doentes MAS. Por fim, pretende-se encontrar diferenças significativas na expressão de anticorpos ou polimorfismos genéticos que possam ser usados como marcadores de poliautoimunidade e permitir a implementação de estratégias preventivas.

Métodos

Os doentes foram selecionados das coortes de SLE, SjS e APS da Unidade de Imunologia Clínica (UIC) – Centro Hospitalar do Porto. Critérios de inclusão: diagnóstico de pelo menos um dos *chaperones* sistémicos (SLE ou SjS ou APS). Critérios de exclusão: menores de 18 anos; diagnóstico de apenas duas DAIs.

Os doentes foram recrutados por telefone e a colheita de sangue sincronizada com marcações prévias. Os dados clínicos e imunológicos foram colhidos por consulta dos processos médicos dos doentes. A genotipagem foi realizada no Laboratório de Imunogenética do Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto. As frequências obtidas do HLA-DRB1 e PTPN22 foram comparadas com as de

uma população controlo. Os dados foram analisados com recurso ao software da IBM SPSS23®.

Resultados

O recrutamento resultou numa população de 331 doentes de ascendência Europeia, cuja distribuição é apresentada na Figura 1. O número de doentes que apresenta cada uma das DAI *chaperones* sistémicas pode ser visto na Figura 2. Estavam presentes 26 outras DAIs (Apêndice 2).

As frequências alélicas do HLA classe II na coorte do estudo apresentavam diferenças relativamente à população controlo, como mostra a Tabela I. As frequências alélicas do PTPN22 eram também significativamente discrepantes da população controlo – Tabela II (o alelo T corresponde ao polimorfismo rs2476601 supracitado).

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A distribuição do grupo SLE está representada na Figura 3.

As frequências alélicas para o grupo SLE e os seus subgrupos mono e MAS são apresentadas na Tabela IV; a Tabela V apresenta as frequências alélicas para o PTPN22 (rs2476601). Ambas as tabelas estabelecem comparação com as frequências alélicas da população controlo.

No grupo SLE, a comparação entre doentes monoautoimunes e MAS surtiu algumas diferenças - Tabela VI. Após análise de potenciais factores confundidores, a elevada frequência das seguintes variáveis clínicas e imunológicas pôde ser atribuída ao MAS (e não a uma DAI individual que possa coexistir): atingimento hematológico, fenómeno de Raynaud, anti-CCP. Dadas as diferenças citadas, procurou-se compreender se estas seriam justificáveis por variáveis imunológicas ou genéticas – a Tabela VI apresenta também estes os possíveis factores de risco e protecção.

Síndrome de Sjögren

A distribuição do grupo SjS está representada na Figura 4.

As frequências alélicas para o grupo SjS e os seus subgrupos mono e MAS são apresentadas na Tabela VII; a Tabela VIII apresenta as frequências alélicas para o PTPN22 (rs2476601). Ambas estabelecem comparação com as frequências alélicas da população controlo.

No grupo SjS, a comparação entre doentes monoautoimunes e MAS surtiu algumas diferenças – Tabela IX. Não foram encontradas diferenças significativas nos polimorfismos genéticos. Após análise de potenciais factores confundidores, nenhuma das diferenças pôde ser exclusivamente atribuída ao MAS. Dadas as diferenças citadas,

procurou-se compreender se estas seriam justificáveis por variáveis imunológicas ou genéticas - a Tabela IX apresenta também estes possíveis factores de risco e protecção.

Síndrome antifosfolípido

A distribuição do grupo APS está representada na Figura 5.

As frequências alélicas para o grupo APS e os seus subgrupos mono e MAS são apresentadas na Tabela X; a Tabela XI apresenta as frequências alélicas para o PTPN22 (rs2476601). Ambas as tabelas estabelecem comparação com as frequências alélicas da população controlo.

No grupo APS, a comparação entre doentes monoautoimunes e MAS surtiu algumas diferenças - Tabela XII. Não foram encontradas diferenças significativas nos polimorfismos genéticos. Após análise de potenciais factores confundidores, apenas o anti-U1RNP e o factor reumatóide foram atribuídos exclusivamente ao MAS. Dadas as diferenças citadas, procurou-se compreender se estas seriam justificáveis por variáveis imunológicas ou genéticas - a Tabela XII apresenta também estes possíveis factores de risco e protecção.

MAS triplos positivos sistémicos

A distribuição do grupo MAS está representada na Figura 6.

As frequências alélicas para o grupo MAS e os seus subgrupos triplo positivo sistémico e outros MAS são apresentadas na Tabela XIII; a Tabela XIV apresenta as frequências alélicas para o PTPN22 (rs2476601). Ambas as tabelas estabelecem comparação com as frequências alélicas da população controlo.

No grupo MAS, a comparação entre doentes triplos positivos sistémicos e outros MAS surtiu algumas diferenças - Tabela XV. Não foram encontradas associações significativas entre variáveis genéticas ou imunológicas e variáveis clínicas.

Dadas as diferenças citadas, procurou-se compreender se estas seriam justificáveis por variáveis imunológicas ou genéticas - a Tabela XV apresenta também estes possíveis factores de risco e protecção.

PTPN22

Na coorte do estudo, o PTPN22_T é factor de risco para expressão de anti-dsDNA e de protecção contra expressão de anti-La/SSB. No grupo SLE, o PTPN22_T tem papel protector contra expressão de aCL IgM no subgrupo MAS e contra LAC no grupo global. Por outro lado, é factor de risco para atingimento gastrointestinal no grupo mono. No grupo SjS, o PTPN22_T tem papel protector contra expressão de anti-Ro/SSA no subgrupo mono. É, no entanto, factor de risco para atingimento pulmonar no grupo SjS,

risco para consumo de C3 e expressão de anti-CCP no subgrupo MAS e risco para expressão de anti-peroxidase no subgrupo mono. No grupo APS o PTPN22_T tem papel protector contra expressão de aCL IgM no global e contra LAC no subgrupo MAS, mas estes estarão, muito provavelmente, associados ao SLE e não ao APS.

Tempo de follow-up

Considerando as diferenças clínicas nos grupos em estudo, dividiram-se os doentes em subgrupos de 5 anos de follow-up, procurando determinar o ponto temporal de surgimento das divergências.

Grupo SLE:

- NPSLE: maior prevalência no subgrupo MAS apenas a partir do segundo intervalo (5-9 anos); em doentes com menos de 5 anos de follow-up, NPSLE é mais frequente no subgrupo mono (sem significado estatístico);
- Mucocutâneo subagudo: mais frequente no subgrupo MAS apenas em doentes com mais de 5 anos de follow-up; no primeiro intervalo, o subgrupo mono tem uma maior frequência de casos (sem significado estatístico);
- Musculoesquelético musculo-tendinoso: sempre mais frequente no subgrupo MAS (diferenças apenas se significativas após 10 anos de follow-up);
- Hematológico: sempre mais frequente no subgrupo MAS;
- Fenómeno de Raynaud: em doentes com menos de 5 anos de follow-up, é mais frequente no subgrupo mono; após os 5 anos, é mais frequente no subgrupo MAS (diferença significativa apenas após 10 anos de follow-up).

Grupo SjS:

- Hipertrofia parotídea: sempre mais frequente no subgrupo mono;
- Crioglobulinemia: em doentes com menos de 5 anos de follow-up é mais frequente no subgrupo mono (sem significado estatístico); em doentes com mais de 5 anos de follow-up é mais frequente no subgrupo MAS;
- Hipocomplementemia: globalmente mais frequente no subgrupo MAS; no entanto, em doentes com 10-14 anos e mais de 20 anos de follow-up é mais frequente no subgrupo mono (sem significado estatístico);
- Fenómeno de Raynaud: sempre mais frequente no subgrupo MAS.

Grupo APS:

- Manifestações não trombóticas: global – em doentes com menos de 5 anos de follow-up a frequência é idêntica; após os 5 anos é mais frequente no subgrupo MAS; fenómeno de Raynaud – sempre mais frequente no subgrupo MAS.

Discussão

O presente estudo confirma o HLA-DRB1*03 como importante factor de risco para DAIs, nomeadamente para SLE e SjS. Confirma também a hipótese recente que sugere que o HLA-DRB1*13 poderá ter um papel protector da autoimunidade[12] e acrescenta uma possível associação com o alelo HLA-DRB1*14, também como protector.

O HLA-DRB1*11 foi descrito como possível factor protector de SLE numa população da América Latina.[6] Na nossa população de ascendência Europeia este alelo é protector contra DAIs em geral e é também um factor protector de SjS; não apresenta, no entanto, significado estatístico no subgrupo mono do SLE. A frequência alélica do HLA-DRB1*01 era também baixa na nossa coorte. Autores sugerem que este alelo poderá ter um papel protector de SjS[36] mas não encontrámos diferenças significativas no subgrupo mono SjS. A frequência do alelo HLA-DRB1*09 na nossa coorte é muito baixa quando comparada com a da população controlo; no entanto, este facto deve ser interpretado cautelosamente, uma vez que a frequência do HLA-DRB1*09 na população controlo em questão é significativamente superior à reportada noutras coortes da mesma área geográfica.[55]

No grupo SLE, o alelo HLA-DRB1*16 tem uma frequência elevada apenas no subgroupo MAS. Apesar de não ter sido encontrada uma associação entre este alelo e SjS, há referências ao mesmo como potencial factor de risco[36] e isso poderia justificar este aumento. O facto de este alelo ser também factor de risco para anti-Ro/SSA reforça essa hipótese.

No grupo SjS, o alelo HLA-DRB1*15 tem frequência elevada e pode, como tal, ser considerado factor de risco. O papel deste alelo no SjS primário já foi reportado[56], o que está em concordância com as nossas observações no subgroupo mono SjS.

Relativamente ao APS, o nosso estudo não confirma os dados de investigações que atribuem um papel de susceptibilidade aos alelos HLA-DRB1*04 e *07,[34, 57-59] nem encontra qualquer factor de risco ou protecção significativo.

Apesar de confirmar o possível papel protector do HLA-DRB1*13 na DAI, o presente estudo encontrou uma associação com a expressão de anticorpos antifosfolípido em doentes MAS de vários grupos. Esta associação poderá apontar para o HLA-DRB1*13 como marcador de risco vascular em doentes com MAS.

Não há ainda consenso sobre a influência da poliautoimunidade na gravidade da doença, com alguns estudos a reportar um curso mais severo[67-69] enquanto outros concluem que terá um efeito protector.[70-73] No presente estudo, os subgrupos MAS apresentam atingimento orgânico mais grave.

O anti-U1RNP no presente estudo parece assumir-se como factor de risco para MAS. É também o único anticorpo que, transversalmente, se encontra associado ao MAS,

sendo defensável que possa ter um papel como marcador de doenças do tecido conjuntivo e possa ser usado para determinar a probabilidade de desenvolvimento de uma segunda doença do tecido conjuntivo em doentes com uma DAI já estabelecida. O alelo HLA-DRB1*15, factor de risco para SjS no presente estudo, é também factor de risco para anti-U1RNP.

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A frequência do alelo HLA-DRB1*07 é significativamente inferior no subgrupo MAS, sugerindo um papel protector contra a multiautoimunidade.

O papel da expressão de anticorpos no SLE neuropsiquiátrico (NPSLE), apesar de extensamente estudado, tem resultados controversos.[75] O presente estudo confirma a associação entre aPL e aCL e NPSLE e, contrariamente aos estudos prévios[75], encontrou uma associação positiva entre aβ2GPI (ainda que nunca no subgrupo mono) e NPSLE. Não foi encontrada qualquer associação com LAC. A elevada frequência de aPLs nos doentes MAS poderá representar uma população no espectro do APS, mas sem claros critérios clínicos trombóticos.

Estudos recentes encontraram uma associação entre anti-U1RNP no líquido cefalo-raquidiano e NPSLE, apesar da ausência de associação com os níveis séricos do anticorpo.[76-78] No presente estudo, no entanto, o anti-U1RNP sérico tem um papel protector de NPSLE central focal no subgrupo monoautoimune.

Vários autores descrevem associações entre anti-Sm e envolvimento orgânico e actividade de doença, com resultados contraditórios que se estendem ao NPSLE.[79] No presente estudo, o anti-Sm tem um papel protector de NPSLE, no grupo SLE em geral e no subgrupo mono SLE em particular.

Há uma associação comummente aceite entre anticorpos anti-Ro/SSA e SLE cutâneo subagudo.[65] O presente estudo confirma essa associação positiva no grupo SLE (global e subgrupo MAS). No subgrupo MAS, o alelo HLA-DRB1*16 é factor de risco para expressão de anti-Ro/SSA e ambos são factores de risco para lesões cutâneas subagudas. Como supracitado, o HLA-DRB1*16 é considerado um possível factor de risco para SjS, o que poderá corroborar as nossas conclusões.

Vários estudos debatem o efeito do anti-C1q no atingimento renal no SLE[80]; no entanto, não parece haver nenhum estudo a reportar uma associação entre este anticorpo e lesões cutâneas subagudas ou NPSLE. No presente estudo, o anti-C1q é factor de risco para o primeiro e de protecção para o último.

Síndrome de Sjögren

O presente estudo encontrou uma associação positiva entre anti-centrómero e crioglobulinemia no subgrupo MAS. Há uma clara associação entre anti-centrómero e fenómeno de Raynaud[81-82], conclusão que o presente estudo não replica. Tal pode dever-se ao reduzido número de doentes com anti-centrómero positivo ou ao facto de o fenómeno de Raynaud ser transversal a diversas DAIs, com múltiplas causas possíveis. O presente estudo encontra também uma associação entre fenómeno de Raynaud e anti-U1RNP, uma associação previamente estabelecida em doenças do tecido conjuntivo, como DMTC[83-85] e SLE[86].

MAS triplos positivos sistémicos

Doentes com SLE, SjS e APS parecem ter maior risco de desenvolver nefrite lúpica grave, comparativamente a outros doentes MAS. É possível que estes eventos não sejam totalmente independentes, uma vez que aPLs e eventos trombóticos podem contribuir para agravar a nefrite lúpica.[87] Simultaneamente, parece haver um maior risco de eventos trombóticos, nomeadamente do sistema nervoso central. O MAS triplo positivo sistémico poderá, portanto, representar um estado pró-trombótico mais grave. Tudo isto são, no entanto, conjecturas, uma vez que este é, segundo sabemos, o primeiro estudo a debruçar-se sobre esta associação específica de DAIs.

Tempo de follow-up

Não existe consenso sobre o tempo de follow-up necessário para diferenciar os doentes monoautoimunes daqueles com potencial para a poliautoimunidade. Por vezes é difícil determinar o intervalo temporal que medeia o início de duas DAIs sucessivas, devido a atrasos no diagnóstico e a subfenótipos comuns a várias DAIs. Estas sobreposições levam frequentemente a uma atribuição errónea de novos sintomas à DAI previamente estabelecida, em detrimento do diagnóstico de uma nova entidade nosológica. A consequente sobrestimação da data de início de sintomas constitui um importante viés em estudos retrospectivos como o presente.

Apesar destas dificuldades, os achados do presente estudo permitem concluir que a diferença entre doentes puramente monoautoimunes e doentes MAS se estabelecerá 5 a 10 anos após o diagnóstico.

PTPN22

Vários estudos procuraram determinar o papel do PTPN22 como factor de susceptibilidade para DAIs.[1, 15, 39-48, 88-89] Este parece ser o primeiro estudo a concluir que o polimorfismo (rs2476601), apesar de mais frequente nos doentes autoimunes,

poderá estar associado a um curso de doença menos grave. Na presente coorte constitui factor de protecção contra expressão de aCL IgM, LAC e anti-Ro/SSA, o que poderá traduzir-se num prognóstico mais benigno em determinados doentes. No entanto, é também factor de susceptibilidade para certos tipos de atingimento de órgão, pelo que mais estudos serão necessários para clarificar o seu papel.

Conclusão

Este parece ser um dos poucos estudos a focar-se nas três dimensões do MAS: atingimento orgânico, expressão imunológica e polimorfismos genéticos. Concluímos que a coexistência de DAIs contribui para um curso de doença mais severo.

O presente estudo confirmou várias associações previamente estabelecidas entre polimorfismos genéticos e autoimunidade, fortalecendo a sua definição como factores de risco ou protecção. Acreditamos que o alelo HLA-DRB1*07 poderá ser usado como marcador de monoautoimunidade, permitindo a estratificação de doentes com uma DAI em termos de probabilidade de desenvolvimento de DAIs adicionais. De forma similar, o anti-U1RNP poderá ser usado como marcador imunológico para o desenvolvimento de uma segunda doença do tecido conjuntivo em doentes com uma DAI já estabelecida.

A associação positiva do HLA-DRB1*13 com aPLs nos doentes MAS poderá conduzir ao uso deste alelo como predictor de risco vascular em doentes com múltiplas DAI.

O papel do polimorfismo (rs2476601) do PTPN22 na autoimunidade é conhecido mas não completamente compreendido. Apesar de ser claramente mais frequente em doentes autoimunes, o presente estudo sugere que poderá estar associado a um curso de doença mais ligeiro. Este achado abre novas possibilidades que carecem de confirmação noutras coortes.

A coexistência dos três chaperones sistémicos da autoimunidade parece criar um estado pró-trombótico, com risco acrescido de trombose do SNC e atingimento renal grave. Novos estudos são necessários para a adequada compreensão desta relação aparentemente sinérgica e suas implicações.

Por último, conclui-se que a divergência entre doentes monoautoimunes e doentes com MAS surgirá 5 a 10 anos após o diagnóstico inicial. Esta poderá ser uma importante conclusão para a prevenção de vieses – para comparar adequadamente doentes mono e multiautoimunes, poderá ser aconselhável selecionar uma população de doentes com mais de 5 anos de diagnóstico.

Apesar das múltiplas associações encontradas, a maioria é exploratória e carece de confirmação em coortes maiores e em doentes de diferentes etnias