ORIGINAL PAPER

Clinical features and genetic biomarkers associated with different phenotypes of systemic lupus erythematosus in Paraguayan patients

I. Acosta-Colman[1], Z. Morel[1], A. Ayala Lugo[2], V. Jolly[2], I. de Guillén[3], P. Langjahr[3], M. Vazquez[1], M.T Martínez de Filártiga[4], M.E. Acosta[3]

[1]Department of Rheumatology, Faculty of Medical Sciences, National University of Asunción, San Lorenzo;
[2]Molecular Genetics Laboratory, Health Sciences Research Institute, National University of Asunción, San Lorenzo;
[3]Production Laboratory, Heath Sciences Research Institute, National University of Asunción, San Lorenzo;
[4]Curie Laboratory, Asunción, Paraguay

SUMMARY

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by a heterogeneous clinical picture that makes the diagnosis and follow-up of these patients difficult. This study aimed to identify correlations between clinical, immunological, and genetic biomarkers and clinical manifestations in SLE.

A retrospective study of data from medical records and immunological and genetic studies of SLE patients in Paraguay was carried out. A descriptive analysis was performed based on the type of variable. Human leukocyte antigen (HLA) allele frequencies (*DPA1*, *DPB1*, *DQA1*, *DQB1*, and *DRB1*) were calculated, and univariate logistic regression analyses were performed between each of the explanatory variables and the presence or absence of each phenotype. Odds ratios, 95% confidence intervals, and p values were recorded. Associations with $p<0.05$ were considered statistically significant. 104 SLE patients were included: 86% were female, with a mean age of 32.80±10.36 years. An association was identified between anti-double stranded DNA (anti-dsDNA) and the presence of the renal phenotype and between anti-dsDNA and the absence of the joint and hematological phenotypes. Immunoglobulin M isotype rheumatoid factor was associated with the absence of a renal phenotype. *HLA-DQB1**02:02 and *HLA-DRB1**07:01 were associated with the cutaneous phenotype. An association was identified between age at disease onset over 30 years and the presence of the joint phenotype. No other associations were identified. Potential clinical, immunological, and genetic biomarkers of phenotypes have been identified in SLE Paraguayan patients.

Key words: *Biomarkers, phenotypes, systemic lupus erythematosus, Paraguayan.*

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic and autoimmune disease, whose etiology is unknown. It is characterized by different immunological alterations, such as loss of self-tolerance, activation of B lymphocytes, and the production of organ-specific and non specific antibodies (1-23). Various research groups on SLE have focused their research on identifying and validating biomarkers that can accurately predict not only susceptibility to suffering from this disease but also its specific phenotypes and activity thereof. These biomarkers would allow health professionals to choose the best-individualized therapy for each SLE patient (4, 8). In relation to the clinical biomarkers of phenotypes in SLE, this study is complex due to the wide spectrum of manifestations that patients may present. However, various cohorts such as the *Lupus in minorities: nature vs nurture* (9-11) and *Latin American Group for the Study of Lupus* (12-14) observed that the age of onset of the disease, sex, and other clinical variables could be biomarkers related to phenotypes and specific pathogenic mechanisms of SLE (14).

Autoantibodies have also been studied on

Corresponding author:
Isabel Acosta-Colman
Department of Rheumatology,
Faculty of Medical Sciences,
National University of Asunción,
San Lorenzo, Paraguay
E-mail: dr.acostacolman@gmail.com

numerous occasions as biomarkers of activity and/or specific clinical manifestations of the disease (15, 16). The presence of these autoantibodies is associated with specific SLE phenotypes according to various studies (17-19).

Currently, more than 30 *loci* have been described in SLE (5, 8, 20). However, although several genes have been associated with various SLE phenotypes in animal models, SLE in humans is polygenic and can vary according to race, making the study even more complex. There are studies in the European population and isolated studies in the Hispanic population evaluating *loci* of the major histocompatibility complex that have observed an association between certain alleles of these genes and the presence of autoantibodies and specific clinical manifestations (1, 8, 20, 21).

The main objective of this study was to identify associations of clinical, immunological, and genetic biomarkers with clinical manifestations in Paraguayan SLE patients.

MATERIALS AND METHODS

Study population

An observational, analytical, retrospective, cross-sectional study, based on the review of medical records of patients diagnosed with SLE [according to the American College of Rheumatology (ACR) 1997 (22) and Systemic Lupus International Collaborating Clinics Classification Criteria (SLICC) 2012 for systemic lupus erythematosus (23)] who were treated in the Department of Rheumatology of the Hospital de Clínicas from January 2015 to March 2017 was carried out. Non-probabilistic sampling was at discretion.

The variables analyzed were clinical, laboratory, and genetic variables. Clinical variables included age, gender, origin, age at disease onset, clinical manifestations, or phenotypes (*i.e*. patients with lupus nephritis). To define the different clinical manifestations or phenotypes, definitions used in the classification criteria proposed by the ACR and the SLICC group were considered. Laboratory variables comprised antinuclear antibodies (ANA), anti-nuclear ribonucleoprotein (anti-RNP), anti-Ro, anti-La, anti-Smith antibodies (anti-Sm), anti-double-stranded DNA (anti-dsDNA), rheumatoid factor (RF), and others. Genetic variables included typing of alleles of human leukocyte antigen (HLA) class II genes: *HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DPA1,* and *HLA-DPB1.*

The identity of the individuals who were the source of the data was safeguarded, complying with confidentiality, as stipulated in the code of professional ethics. This study was approved by the Ethics Committee of the Hospital. The patients signed the respective informed consent for the use of their clinical data and for the collection of biological material.

Assessment of human leukocyte antigen genotype

To determine the genotype, 8 mL of peripheral blood was extracted from each individual. Starting from 2 mL, the genomic DNA extraction and purification process was carried out using the Purelink column system (Life-technologies®, Carlsbad, USA). The typing of the alleles of the HLA class II genes (*HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DPA1* and *HLA-DPB1*) was carried out using the Luminex® xMAP™ technology (Austin, USA) at the Curie Laboratory. This technology determines the alleles of each gene using different sequence-specific oligonucleotide probes attached to microspheres labeled with different intensities of fluorescence, red and infrared dyes. The commercial Lifecodes kits (Immunocor, USA) were used.

The descriptive analysis was performed at global (all patients) and individual levels for each phenotype studied. For all variables, the number and percentage of valid values are shown, and the descriptive statistic is calculated based on their type: for numerical variables, the mean and standard deviation, for binary/dichotomous variables, the number and percentage of positive values, and for categorical variables the number and percentage of patients for each of the possible values. After the descriptive analysis, a univariate logistic regression analysis was performed between each of the explanatory variables (*i.e.* demographic,

epidemiological, clinical, and laboratory variables as independent variables) and the presence/absence of each of the phenotypes (response or dependent variable).

Statistical analyses

Only specific phenotypes with more than 10 patients and variables that were not constant (same value for all patients) have been taken into account. For each phenotype-variable pair analyzed, the odds ratio (OR), the 95% confidence interval (CI 95%), and the p value are provided. In the case of the alleles of the HLA haplotypes, the association test was proposed according to an additive model (0: no allele; 1: 1 allele present; 2: homozygous for the allele considered). For further analysis, only alleles with a global allele frequency above 0.05 or 5% were taken into account. Associations with $p<0.05$ were considered statistically significant. The statistical package R was used for the biological and biomedical analysis, specifically, the genetic analysis that is required for this project.

Ethics approval

This work has been approved by the Ethics Committee of the Faculty of Medical Sciences of the National University of Asunción (UNA_FCM_DI Nº 94/2017).

RESULTS

In this study, 104 SLE patients were included: 86% were female, with a mean age of 32.80±10.36 years, and 97% came from urban areas (Table I).

Eight phenotypes were identified, the most frequent being renal in 49% (51/104) of patients, followed by articular in 29% (30/104) patients, and cutaneous in 27% (28/104) patients. In relation to the coexistence of a phenotype, it was observed that 34% (35/104) of the patients presented more than one phenotype. In this regard, 23.5% (12/51) of patients with renal phenotype also presented cutaneous phenotype and 55% of patients (11/20) with hematological phenotype also had other minor phenotypes. Among the patients with articular phenotype, 30% presented a cutaneous phenotype and 10% a renal phenotype. In 33% of patients with a neurological phenotype, a renal phenotype was identified (Table II).

In relation to the age of the onset by phenotype, it was observed that the renal phenotype predominated in patients who started the disease before 30 years of age and the articular phenotype predominated in those who started it after 30 years (OR=1.046; CI 95%=1.00-1.09; p=0.044).

All patients presented positive ANA. Regarding the profile of antibodies identified, the most frequently identified antibody was anti-dsDNA in 48.1% (50/104) of patients, followed by anti-RNP in 29% (28/95), anti-Ro with 28.7% (29/104), anti-Sm with 15.5% (15/97), and, less frequently, anti-topoisomerase I (anti-Scl70), anti-histidyl-ARN-t- synthetase (anti-Jo 1) and anti-La. In the renal phenotype, positive anti-dsDNA predominated in 86.3% (44/51), followed by anti-RNP in 34% (17/50), anti-Ro in 29.4% (15/51) and anti-Sm in 18.36% (9/49). In the articular phenotype, anti-Ro predominated in 39.3% (11/28) of patients and anti-RNP in 32% (8/25). In the cutaneous phenotype, anti-dsDNA predominated in 46.4% (14/28) of patients, followed by anti-Ro with a 33.3% (9/27). Anti-dsDNA predominated in the hematological phenotype in 25% (5/20) of patients. In the pulmonary phenotype, anti-Ro predominated in 40% (2/5), anti-RNP in 40% (2/5), and anti-ds DNA in 33% (2/6) of patients. Regarding the positivity of the antibodies, it was observed that more than 80% of the patients who showed positive anti-dsDNA presented a renal phenotype followed by a phenotype with cardiac and cutaneous involvement, as observed in Figure 1.

RF measurement [total, immunoglobulin (Ig) A, IgG, IgM] was also performed. Total RF was positive in 21.4% of patients (21/98), IgA positive in 5.1% (5/98), IgG positive in 21.45% (21/98), and IgM positive in 15.3% (15/98). The pulmonary phenotype was observed more frequently associated with RF, IgG and IgM.

In order to describe HLA alleles and evaluate their association with the phenotypes studied, the haplotype profiles for HLA class II genes (*HLA-DPA1*, *HLA-DPB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DRB1*)

Table I - Clinical, biological and demographic characteristics of the study population.

Demographic characteristics	Frequency (%)	Mean (SD)	Total (N)
Current age		32.80 (10.36)	104
Age of onset of symptoms		27.32 (10.50)	85
Sex	14(13.4%) / 90 (86.5%)		104
Immunological profile	**Frequency (%)**	**Mean (SD)**	**Total (N)**
Rheumatoid Factor	21 (21.4%)		98
Rheumatoid Factor IgG	21 (21.4%)		98
Rheumatoid Factor IgM	15 (15.3%)		98
Rheumatoid Factor IgA	5 (5.1%)		98
ANA	Positive (100%)		104
C3 low	14 (14.4%)	137 (48)	97
C4 low	41 (42.2%)	22.48 (12.28)	97
Anti-Sm	15 (15.5%)		97
Anti-DNA	50 (48.1%)		104
Anti-SSA	29 (28.7%)		101
Anti-SSB	5 (5.1%)		99
Anti-RNP	28 (29.5%)		95
Anti-Scl70	1 (1%)		99
Anti-Jo 1	5 (5.3%)		95
Clinical phenotypes	**Frequency (%)**	**Mean (SD)**	**Total (N)**
Renal	51 (32.9%)		155
Articular	30 (19.3%)		155
Cutaneous	28 (18%)		155
Hematological	20 (12.9%)		155
Pulmonary	6 (3.8%)		155
Cardiac	4 (2.5%)		155
Neurological	3 (2%)		155
Other	13 (8.3%)		155

SD, standard deviation; N, number; IgG, immunoglobulin G; IgA, immunoglobulin A; ANA, antinuclear antibodies; Anti-SSA, anti-Sjögren's syndrome related antigen A; Anti-SSB, anti-Sjögren syndrome antigen B; Anti-RNP, antinuclear ribonucleoprotein; anti-Scl70, anti-topoisomerase I; Anti-Jo 1, anti-histidyl-ARN-t- synthetase; Anti-Sm, anti-Smith.

Table II - Correlation of phenotypes identified in the studied patients. The correlation between phenotypes is analyzed at a descriptive level. For each phenotype (line), the percentage of patients who also have each of the other phenotypes (columns) is shown.

	Renal	Articular	Cutaneous	Hematological	Pulmonary	Cardiac	Neurological	Other
Renal	*100% (51/51)*	5.9% (3/51)	23.5% (12/51)	7.8% (4/51)	0% (0/51)	5.9% (3/51)	2% (1/51)	2.0% (1/51)
Articular	10% (3/30)	*100% (30/30)*	30% (9/30)	0% (0/30)	10% (3/30)	0% (0/30)	3.3% (1/30)	6.7% (2/30)
Cutaneos	42.9% (12/28)	32.1% (9/28)	*100% (28/28)*	7.1% (2/28)	3.6% (1/28)	3.6% (1/28)	0% (0/28)	3.6% (1/28)
Hematological	20% (4/20)	0% (0/20)	10% (2/20)	*100% (20/20)*	5% (1/20)	5% (1/20)	5% (1/20)	55% (11/20)
Pulmonary	0% (0/6)	50% (3/6)	16.7% (1/6)	16.7% (1/6)	*100% (6/6)*	0% (0/6)	16.7% (1/6)	0% (0/6)
Cardiac	75% (3/4)	0% (0/4)	25% (1/4)	25% (1/4)	0% (0/4)	*100% (4/4)*	0% (0/4)	0% (0/4)
Neurological	33.3% (1/3)	33.3% (1/3)	0% (0/3)	33.3% (1/3)	33.3% (1/3)	0% (0/3)	*100% (3/3)*	33.3% (1/3)
Other	7.7% (1/13)	15.4% (2/13)	7.7% (1/13)	84.6% (11/13)	0% (0/13)	0% (0/13)	7.7% (1/13)	*100% (13/13)*

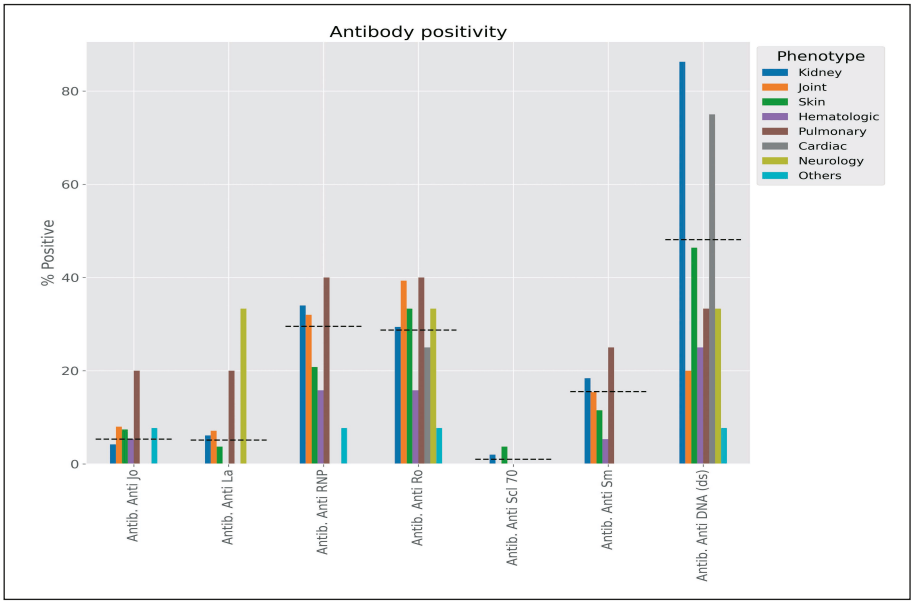

Figure 1 - Phenotype according to antibody positivity.
Anti SSA, anti-Sjögren's syndrome related antigen A; Anti SSB, anti-Sjögren syndrome antigen B; Anti RNP, antinuclear ribonucleoprotein; Anti Scl70, anti-topoisomerase I; Anti Jo 1, anti-histidyl-ARN-t- synthetase; Anti Sm, anti-Smith.

were determined (Figure 2). Of the 5 genes studied, only the *HLA-DQA1* gene was found in Hardy-Weinberg imbalance ($p<0.05$), which should be considered when interpreting the results. In the *HLA-DPA1 locus*, 14 alleles were identified, of which *HLA-DPA1**01:03 and 01:02 were the most frequently identified with an allele frequency of 64% and 25%, respectively. The other alleles of this *locus* presented with a frequency of 6 to 0.5%. Regarding the *HLA-DPB1 locus*, 24 alleles were identified; the most frequent alleles were *HLA-DPB1**04:01, *HLA-DPB1**04:02 and *HLA-DPB1**03:01 with an allele frequency of 26%, 16%, and 11%, respectively. In the *HLA-DQA1 locus*, 11 alleles were identified, of which the most frequent were *HLA-DQA1**01:02 and *HLA-DQA1**03:01 with an allele frequency of 22% and 13%, respectively. The other alleles presented a frequency between 0.5 and 12%. 18 alleles were identified at the *HLA-DQB1 locus* with a frequency ranging from 0.5 to 14%. Finally, in the *HLA-DRB1 locus*, 43 alleles were identified with a frequency ranging from 0.5 to 13%, with the most frequent allele being *HLA-DRB1**07:01 followed by *HLA-DRB1**15:01. Regarding *HLA-DQB1*, it was observed that with the *HLA-DQB1* 02:02 allele there was a statistically significant association and a risk of developing the cutaneous phenotype (OR=2.52; CI 95%=1.05-6.97; p=0.038). Regarding *HLA-DRB1*, the *HLA-DRB1* 07:01 allele presented a statistically significant association and a risk of presenting the cutaneous phenotype (OR=2.49; CI 95%=1.06-5.86; p=0.035). No other statistically significant association or risk was found with the other alleles analyzed (Figure 3).

A significant association and risk were found between the presence of anti-dsDNA and the renal phenotype (OR=49.23; CI 95%=15.35-157.91; p=0.00001) and a statistically significant protection factor was found for both the hematological (OR=0.28; CI 95%=0.09-0.86; p=0.026) and the articular (OR=0.17; CI 95%=0.06-0.46; p=0.001) phenotypes. Another significant association and protective factor were observed between the presence of RF IgM and the renal phenotype (OR=0.201; CI 95%=0.05-0.76; p=0.019) (Table III).

DISCUSSION

In this study, we describe the characteristics of SLE patients in Paraguay. The clinical manifestations of SLE show geographic and/or ethnic variations between populations (24). The epidemiological characteris-

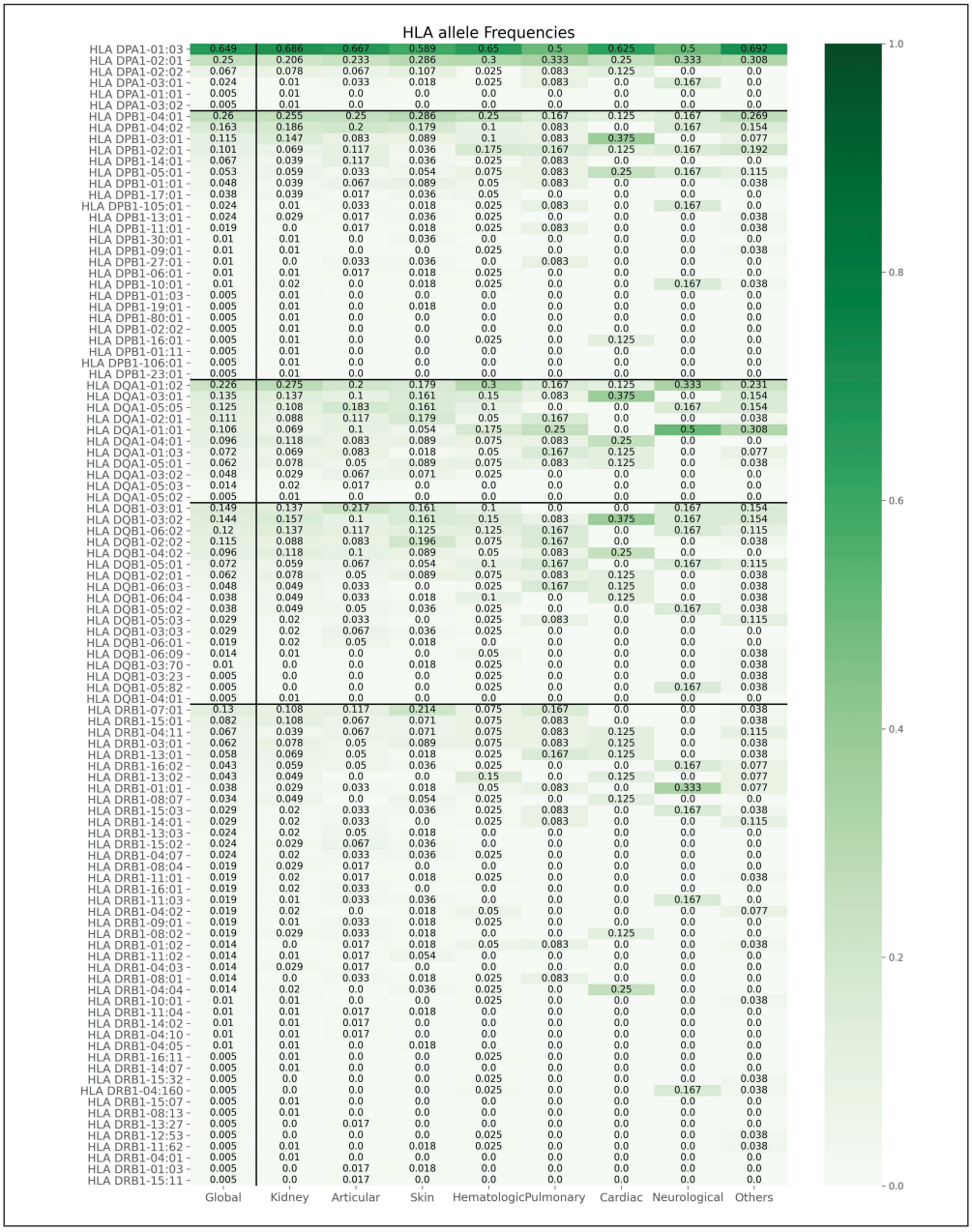

Figure 2 - Heat map with allele frequencies, globally and for each phenotype. The allele frequency is represented by color and in text in each of the cells corresponding to a certain allele for a certain phenotype.

tics found in this study were similar to those reported in other cohorts in terms of sex and age at disease onset (24-26).

Hispanics, Afro-descendants, and Asians develop renal, hematologic, serologic, neuropsychiatric, and immunologic abnormalities more frequently than Caucasians (24, 26). Lupus nephritis (LN), the most worrisome manifestation of SLE, is significantly more frequent in them (24-27). This coincides with our study, which included the Hispanic mestizo population, where we observed that the most frequent identified phenotype was renal. The disease typically presents at a younger age in non-Caucasians (Hispanics, African descent, and Asians) with a more rapid ac-

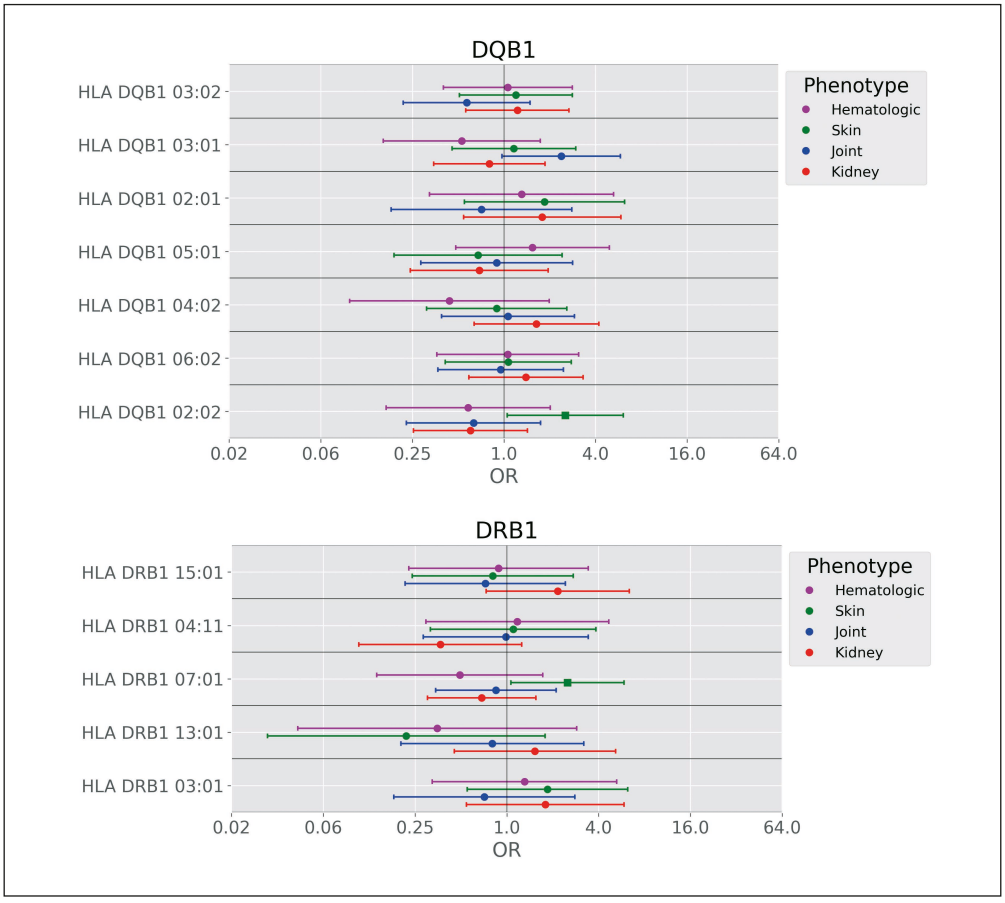

Figure 3 - Odds ratios (OR) for the presence of alleles of HLA-DQB1 and HLA-DRB1 gene and the development of the different phenotypes analyzed.

Table III - Analysis of associations and odds ratios for the presence of autoantibodies and the phenotypic manifestations of systemic lupus erythematosus.

Auto antibodies	Articular OR (CI 95%); p	Cutaneous OR (CI 95%); p	Hematologic OR (CI 95%); p	Renal OR (CI 95%); p
Anti-dsDNA	0.17 (0.06-0.46); p=0.001	0.91 (0.38-2.17); p=0.838	0.28 (0.09-0.86); p=0.027	49 (15-157); p=0.00001
Anti-Sm	0.99 (0.28-3.44); p=0.99	0.64 (0.16-2.48); p=0.52	0.25 (0.03-2.06); p=0.2	1.57 (0.51-4.82); p=0.42
Anti-SSA	0.99 (0.28-3.44); p=0.98	0.64 (0.16-2.48); p=0.52	0.25 (0.03-2.06); p=0.19	1.57 (0.51-4.82); p=0.42
Anti-RNP	1.76 (0.43-3.15); p=0.747	0.54 (0.18-1.65); p=0.287	0.38 (0.10-1.43); p=0.15	1.59 (0.64-3.90); p=0.30
RF total	1.06 (0.36-3.11); p=0.906	0.58 (0.17-1.94); p=0.38	1.96 (0.64-6.02); p=0.235	0.41 (0.15-1.14); p=0.09
RF IgG	1.06 (0.36-3.11); p=0.906	0.58 (0.17-1.94); p=0.38	1.96 (0.64-6.02); p=0.235	0.41 (0.15-1.14); p=0.09
RF IgM	1.96 (0.62-6.18); p=0.247	0.65 (0.16-2.52); p=0.53	2.46 (0.72-8.32); p=0.14	0.20 (0.05-0.76); p=0.019

OR, odds ratio; CI, confidence interval; anti-dsDNA, anti-double-stranded DNA antibodies; Anti-Sm, anti-Smith; anti RNP, anti nuclear ribonucleoprotein; RF, rheumatoid factor; Anti SSA, anti-Sjögren's syndrome related antigen A; IgG, immunoglobulin G; IgA, immunoglobulin A. Phenotypes and variables with more than 10 individuals were included in the analysis.

cumulation of damage and a higher mortality rate. In our study, the phenotypes that predominated in patients under 30 years of age were the renal and neurological phenotypes, which are considered the clinical manifestations that lead to greater morbidity and mortality in SLE patients (3, 4, 6, 25, 26). Male patients were characterized by frequent presentation of serious phenotypes such as the renal phenotype, similar to the RELESSER cohort (28). SLE is a challenge for clinical management because of its heterogeneous clinical picture and because it can affect one or more organs at the same time or sequentially at any time during the evolution of the disease (29). Therefore, the identification of phenotype biomarkers has been a priority for research groups in recent years.

In this study, a variety of autoantibodies have been identified, the most frequent being anti-dsDNA, followed by anti-RNP and anti-Ro. Anti-dsDNA antibodies have been one of the classic diagnostic criteria for SLE since 1982. In 2012, a high serum anti-dsDNA antibody titer accompanied by biopsy-proven LN was accepted as an independent classification criterion for SLE (23). Anti-ds-DNA antibodies are present in the serum in almost 80% of patients with LN. These antibodies interact directly or indirectly with renal antigens, thus producing immune complexes (30). Similar to these studies, we identified a high percentage of SLE patients with positive anti-dsDNA antibodies, especially those with kidney involvement. As part of the immunological profile study, we included some less common antibodies in SLE such as anti-Jo1 and anti-Scl70 which were observed in a few patients who clearly fulfilled the criteria for lupus. This has already been described in studies of other cohorts, where less traditional antibodies have been identified in SLE patients (31).

Paraguay is located in South America, and the population is mainly made up of mestizos, descendants of the original population, the Guarani, and Europeans. In this study, we did not include the indigenous population, which is well differentiated from the mestizo one, mainly because they live in closed communities identified by their customs; as a result there are currently no records of this type of disease in this specific population.

The most frequently identified HLA alleles (allele frequency greater than 10%) in the Paraguayan patients with SLE were *HLA-DPA1**01:03, *DPA1**01:02, *HLA-DPB1**04:01, *DPB1**04:02, *DPB1**03:01, *HLA-DQA1**01:02, *DQA1**03:01, *HLA-DQB1* 03:01, *DQB1* 03:02 and finally *HLA-DRB1**07:01 and *DRB1**15:01. Of all of them, the *HLA-DPA1 locus* contains the most frequent alleles. Benitez *et al*. studied *HLA-A*, *HLA-B* and *HLA-DR* in Paraguayan mestizo individuals (32). In that study, the most frequently identified *HLA-DR* alleles differ from those found in our work, perhaps due to the genotyping technique used and the sample size of the first study. However, our results coincide with other studies carried out in other cohorts of healthy individuals, such as the one published by Montero-Martín et *al*. which was carried out in a Spanish population, where they identified as most frequent *HLA DPA1**01:03, *DPB1**04:01, *DPB1**04:02, *HLA DQB1**03:01, *DQB1**03:02, *HLA DQA1**01:02 and *HLA DRB1**07:01 and *DRB1**15:01 (33).

In this study, we identified two statistically significant associations between *HLA-DQB1**02:02 and *HLA-DRB1**07:01 and skin phenotype. These alleles have previously been identified in different cohorts as being associated with autoimmune pathologies, such as autoimmune diabetes, celiac disease, and rheumatoid arthritis (34-36). However, this is the first time that they have been identified as specifically associated with a cutaneous phenotype in SLE.

In a meta-analysis published by the group of Niu et al. (37), *HLA-DR3* and *DR15* were associated with an increased risk of suffering from LN. In our study, both alleles have OR>1, suggesting a risk of presenting this phenotype; however, this association was not statistically significant.

Diaz Gallo et al. (38) identified the *HLA-DRB1**03 allele with the presence of a cutaneous phenotype, specifically with discoid lupus, and *HLA-DRB1**15 associated with LN. This is similar to our results since a higher risk of skin phenotype and renal phenotype was observed with these alleles; however, this association was not statistically significant.

In another study, *HLA-DRB1**08 was identified as a risk allele for a neurological phenotype in the Portuguese population (39). In this same study, they did not identify associations between the different HLA risk alleles and the renal phenotype. In our study we did not evaluate the association with this phenotype because it is a group with a small number of patients; we agree with this study in not having identified other associations with the renal phenotype.

In a study including 127 SLE patients, the association of *HLA-DRB1**07 and *DRB1**07-*DQB1**02 haplotypes with joint and lung involvement (40), *DRB1**03 and *DQB1**02 alleles and the *DRB1**03-*DQB1**02 haplotypes with cutaneous and renal involvement, and *DRB1**13 and *DRB1* haplotypes *13-*DQB1**06 with kidney involvement were observed. Our study has identified *HLA-DRB1**07 as associated with a cutaneous phenotype. We did not find an association of any allele with the analyzed joint phenotype, unlike this study that identified this association with the joint and pulmonary phenotype.

In this study, IgM RF was identified as a protective factor for developing a renal phenotype. This is in line with a study that showed that LN patients had a statistically significant higher prevalence of anti-ds DNA antibodies, but a lower prevalence of RF (41). In this study, we found no association with other antibodies, such as anti-Ro, or anti-Sm, which have been previously described as possible markers of clinical phenotypes in other cohorts (42, 43). Age has been identified as a biomarker of phenotypes. Arthritis was predominant in adult-onset SLE (78.5%) compared to late-onset SLE (57.7%) (44). This is similar to our results, which suggest that there is an association between the presence of arthritis and the age of onset over 30 years.

These differences between other studies and ours may be due to the size of our sample (which is the main limitation of our study as it confines the formation of groups with different phenotypes) and to the difference between the ethnic group included in our work and those included in the studies analyzed. However, these results constitute a great contribution to the knowledge of SLE in the Latin American population, in which there are few publications on HLA and SLE phenotypes.

CONCLUSIONS

This study evaluates for the first time the immunological and genetic profile in relation to phenotypes in SLE patients of Paraguayan origin. Possible biomarkers of phenotypes have been identified and information has been generated to be validated by independent cohorts. It is a study that will serve as the basis for future studies at a national and international level due to the important contribution to the knowledge of SLE.

Contributions

IAC, MTMF, MEA, performed the research and designed the research study; ZM, AAL, VJ, IG, MV, contributed essential reagents or tools; IAC, MTMF, MEA, analyzed the data; IAC, ZM, PL, wrote the paper.

Conflict of interest

The authors declare no potential conflict of interest.

Ethics approval and consent to participate

This work was approved by the Ethics Committee of the Faculty of Medical Sciences of the National University of Asunción (UNA_FCM_DI Nº 94/2017).

Patient consent for publication

The patients signed the respective informed consent for the use of their clinical data and for the collection of biological material (blood). The identity of the individuals who were the source of the data was safeguarded, complying with confidentiality, as stipulated in the code of professional ethics.

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Availability of data and materials

Data and materials are available from the corresponding author upon request.

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REFERENCES

1. Peñaranda-Parada E, Quintana G, Yunis JJ, Mantilla R, Rojas W, Panqueva U, et al. Clinical, serologic, and immunogenetic characterization (HLA-DRB1) of late-onset lupus erythematosus in a colombian population. Lupus. 2015; 24: 1293-9.
2. Pons-Estel GJ, Catoggio LJ, Cardiel MH, Bonfa E, Caeiro F, Sato E, et al. Lupus in latinamerican patients: lessos from the GLADEL cohorte. Lupus. 2015; 24: 536-45.
3. Cervera R, Khamashta MA, Font J, Sebastini GD, Gil A, Lavilla P, et al. Systemic lupus erythematosis: clinical and inmmunologic patterns of disease expression in a cohort of 1000 patients. the european working partyon systemic lupus erythematosus. Medicine. 1993; 72: 113-24.
4. Hanly JG, Urowitz MB, Su L, Bae SC, Gordon C, Clarke A, et al. Autoantibodies as biomarkers for the predition of neuropsychiatric events in systemic lupus erythematosus. Ann Rheum Dis. 2011; 70: 1726-32.
5. Bang SY, Choi JY, Park S, Choi J, Hong SJ, Lee HS, et al. Brief report: influence of HLA-DRB1 susceptibility alleles on the clinical subphenotypes of systemic lupus erythematosus in koreans. Arthritis Rheumatol. 2016; 68: 1190-6.
6. Ichinose K, Arima K, Umeda M, Fukui S, Nishino A, Nakashima Y, et al. Predictors of clinical outcomes in patients with neuropsychiatric systemic lupus erythematosus. Cytokine. 2016; 79: 31-7.
7. Zhang H, Chambers W, Sciascia S, Cuadrado MJ. Emerging therapies in systemic lupus erythematous: from clinical trial to the real life. Expert Rev Clin Pharmacol. 2016; 9: 681-94.
8. González LA, Ugarte-Gil MF, Alarcón GS. Systemic lupus erythematosus: the search for the ideal biomarker. Lupus. 2021; 30: 181-203.
9. Burgos PI, McGwin G Jr, Pons-Estel GJ, Reveille JD, Alarcón GS, Vilá LM. US patients of hispanic and african ancestry develop lupus nephritis early in the disease course: data from LUMINA, a multiethnic US cohort (LUMINA LXXIV). Ann Rheum Dis. 2011; 70: 393-4.
10. Danila MI, Pons-Estel GJ, Zhang J, Vilá LM, Reveille JD, Alarcón GS. Renal damage is the most important predictor of mortality within the damage index: data from LUMINA LXIV, a multiethnic US cohort. Rheumatology (Oxford). 2009; 48: 542-5.
11. Burgos PI, McGwin G Jr, Reveille JD, Vilá LM, Brown EE, Alarcon GS. Is familial lupus different from sporadic lupus? Data from LUMINA (LXXIII), a multiethnic US cohort. Lupus. 2010; 19: 1331-6.
12. González-Naranjo LA, Betancur OM, Alarcón GS, Ugarte-Gil MF, Jaramillo-Arroyave D, Wojdyla D, et al. Features associated with hematologic abnormalities and their impact in patients with systemic lupus erythematosus: data from a multiethnic latin american cohort. Semin Arthritis Rheum. 2016; 45: 675-83.
13. Ugarte-Gil MF, Pons-Estel GJ, Molineros J, Wojdyla D, McGwin G Jr, Nath SK, et al. Disease features and outcomes in united states lupus patients of hispanic origin and their mestizo counterparts in latin america: a commentary. Rheumatology (Oxford). 2016; 55: 436-40.
14. Catoggio LJ, Soriano ER, Imamura PM, Wojdyla D, Jacobelli S, Massardo L, et al. Late-onset systemic lupus erythematosus in latin americans: a distinct subgroup? Lupus. 2015; 24: 788-95.
15. Illei G, Tackey E, Lapteva L, Lipsky P. Biomarkers in systemic lupus eritematosus. II markers of disease activity. Arthritis Rheum. 2004; 50: 2048-65.
16. Sarbu MI, Salman-Monte TC, Muñoz R, Lisbona MP, Almiral Bernabe M, Carbonell J. Differences between clinical and laboratory findings in patines with recent diagnosis of SLE according to the positivity of anti-DNA by crithidia luciliae method. Lupus. 2015; 24: 1198-203.
17. Heinlen LD, McClain MT, Merrill J, Akbarali YW, Edgerton CC, Harley JB, et al. Clinical criteria for systemic lupus erythematosus precede diagnosis, and associated autoantibodies are present before clinical symptoms. Arthritis Rheum. 2007; 56: 2344-51.
18. Arbuckle MR, James JA, Kohlhase KF, Rubertone MV, Dennis GJ, Harley JB. Development of anti-dsDNA autoantibodies prior to clinical diagnosis of systemic lupus erythematosus. Scand J Immunol. 2001; 54: 211-9.
19. Heinlen LD, Ritterhouse LL, McClain MT, Keith MP, Neas BR, Harley JB, et al. Ribosomal P autoantibodies are present before SLE onset and are directed against non-C-terminal peptides. J Mol Med. 2010; 88: 719-27.
20. Buniello A, MacArthur JAL, Cerezo M, Harris LW, Hayhurst J, Malangone C, et al. The NHGRI-EBI GWAS catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. Nucleic Acids Res. 2019; 47: D1005-12.
21. Marchini J, Howie B. Genotype imputation for genome-wide association studies. Nat Rev Genet. 2010; 11: 499-511.
22. Hochberg MC. Updating the american college

of rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997; 40: 1725.
23. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012; 64: 2677-86.
24. González LA, Toloza SM, McGwin G Jr, Alarcón GS. Ethnicity in systemic lupus erythematosus (SLE): its influence on susceptibility and outcomes. Lupus. 2013; 22: 1214-24.
25. Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. Medicine. 2003; 82: 299-308.
26. Pons-Estel BA, Catoggio LJ, Cardiel MH, Soriano ER, Gentiletti S, Villa AR, et al. The GLADEL multinational latin american prospective inception cohort of 1,214 patients with systemic lupus erythematosus: ethnic and disease heterogeneity among 'hispanics'. Medicine (Baltimore) 2004; 83: 1-17.
27. Zavala-Miranda MF, Perez-Arias AA, Márquez-Macedo SE, Comunidad-Bonilla RA, Romero-Diaz J, Morales-Buenrostro LE, et al. Characteristics and outcomes of a hispanic lupus nephritis cohort from Mexico. Rheumatology (Oxford). 2023; 62: 1136-44.
28. Riveros Frutos A, Casas I, Rúa-Figueroa I, López-Longo FJ, Calvo-Alén J, Galindo M, et al. Systemic lupus erythematosus in spanish males: a study of the spanish rheumatology society lupus registry (RELESSER) cohort. Lupus. 2017; 26: 698-706.
29. Durcan L, O'Dwyer T, Petri M. Management strategies and future directions for systemic lupus erythematosus in adults. Lancet. 2019; 393: 2332-43.
30. Yung S, Chan TM. Mechanisms of kidney injury in lupus nephritis - the role of anti-dsDNA antibodies. Front Immunol. 2015; 6: 475.
31. Gomard-Mennesson E, Fabien N, Cordier JF, Ninet J, Tebib J, Rousset H. Clinical significance of anti-histidyl-tRNA synthetase (Jo1) autoantibodies. Ann N Y Acad Sci. 2007; 1109: 414-20.
32. Benitez O, Busson M, Charron D. Loiseau P. HLA polymorphism in a guarani-indian population from paraguay and its usefulness for the hispano-indian admixture study in paraguay. Int J Immunogenet. 2011; 38: 7-11.
33. Montero-Martín G, Mallempati KC, Gangavarapu S, Sánchez-Gordo F, Herrero-Mata MJ, Balas A, et al. High-resolution characterization of allelic and haplotypic HLA frequency distribution in a spanish population using high-throughput next-generation sequencing. Hum Immunol. 2019; 80: 429-36.
34. Zhang M, Lin S, Yuan X, Lin Z, Huang Z. HLA-DQB1 and HLA-DRB1 variants confer susceptibility to latent autoimmune diabetes in adults: relative predispositional effects among allele groups. Genes (Basel). 2019; 10: 710.
35. Alshiekh S, Zhao LP, Lernmark Å, Geraghty DE, Naluai ÅT, Agardh D. Different DRB1*03:01-DQB1*02:01 haplotypes confer different risk for celiac disease. HLA. 2017; 90: 95-101.
36. Lagha A, Messadi A, Boussaidi S, Kochbati S, Tazeghdenti A, Ghazouani E, et al. HLA DRB1/DQB1 alleles and DRB1-DQB1 haplotypes and the risk of rheumatoid arthritis in tunisians: a population-based case-control study. HLA. 2016; 88: 100-9.
37. Niu Z, Zhang P, Tong Y. Value of HLA-DR genotype in systemic lupus erythematosus and lupus nephritis: a meta-analysis. Int J Rheum Dis. 2015; 18: 17-28.
38. Diaz Gallo LM, Lundström E, Oke V, Elvin K, Wu YL, Gustafsson J, et al. S4D:6 SLE comprises four immune-phenotypes, which differ regarding HLA-DRB1 and clinical associations. Lupus Sci Med. 2018; 5: doi: 10.1136/lupus-2018-abstract.25.
39. Vasconcelos C, Carvalho C, Leal B, Pereira C, Bettencourt A, Costa PP, et al. HLA in portuguese systemic lupus erythematosus patients and their relation to clinical features. Ann NY Acad Sci. 2009; 1173: 575-80.
40. Rasouli-Saravani A, Tahamoli-Roudsari A, Behzad M, Hajilooi M, Solgi G. Clinical relevance of HLA-DRB1 and -DQB1 alleles in iranian systemic lupus erythematosus patients. Iran J Allergy Asthma Immunol. 2021; 20: 67-75.
41. Drakoulogkona O, Barbulescu AL, Rica I, Musetescu AE, Ciurea PL. The outcome of patients with lupus nephritis and the impact of cardiovascular risk factors. Curr Health Sci. J 2011; 37: 70-4.
42. Fredi M, Cavazzana I, Quinzanini M, Taraborelli M, Cartella S, Tincani A, et al. Rare autoantibodies to cellular antigens in systemic lupus erythematosus. Lupus. 2014; 23: 672-7.
43. Arroyo-Avila M, Santiagos-Casas Y, McGwin G, Cantor R, Petri M, Rmasey-Goldman R et al. Clinical associations of anti-smith antibodies in profile: a multi-ethnic lupus cohort. Clin Rheumatol. 2015; 34: 1217-23.
44. Sassi RH, Hendler JV, Piccoli GF, Gasparin AA, da Silva Chakr RM, Brenol JC, et al. Age of onset influences on clinical and laboratory profile of patients with systemic lupus erythematosus. Clin Rheumatol. 2017; 36: 89-95.