Role of class II human leucocyte antigens in the progression from early to definite systemic sclerosis

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Abstract

Objective. HLAs have been extensively associated with SSc susceptibility but their role in the progression of the disease is poorly understood. In 2013 the ACR and European League Against Rheumatism (EULAR) jointly defined criteria for the classification of SSc that allow the early identification of definite SSc patients. In this study we investigated the role of HLA class II antigens in the progression from early to definite SSc.

Methods. One hundred and fifty-eight subjects with early SSc according to LeRoy and Medsger criteria and no other manifestation indicative of definite SSc at referral were considered. All the patients underwent high-resolution HLA class II typing and the appraisal of definite SSc was retrospectively conducted in a prospective manner. Lifetime analysis was conducted to gauge the effect of genetic and clinical characteristics on progression of the disease.

Results. The median estimated time to progression was 45 months from referral; the 5 and 10 year estimates of progression were 59.8% and 80%, respectively. ACAs were associated with a reduced risk of progression [median survival 55 vs 23 months for ACA-positive vs ACA-negative patients, P = 0.035; hazard ratio (HR) 0.67 (95% CI 0.458, 0.979)]. HLA alleles within the HLA DQ5-DR1 haplotype [HLA-DRB1*0101-HLA-DQA1*0101(4)-HLA-DQB1*0501] reduced the risk of progression of the disease [median survival 108 vs 44 months for DQ5-DR1 carriers vs DQ5-DR1 non-carriers; HR 0.388 (CI 0.211, 0.712), P = 0.001, corrected P = 0.014]. In multivariate models, the effect of genetics was found to be independent of ACA positivity or other baseline factors; additive risks were observed when the DQ5-DR1 haplotype and ACA were jointly considered.

Conclusion. HLA class II alleles within the HLA DQ5-DR1 haplotype are associated with lower rates of progression from early to definite SSc.

Key words: systemic sclerosis, human leucocyte antigens, classification criteria.

Introduction

SSc is a complex autoimmune disorder characterized by vasculopathy and activation of the immune system leading to fibrosis of the skin and internal organs [1]. The production of SSc-specific autoantibodies and the presence of microvascular damage, assessed via nailfold videocapillaroscopy (NVC), are early events in the natural history of SSc that may antedate overt disease by months or years [2]. The importance of these aspects in patients with RP in diagnosing SSc in its earliest stages has long been recognized and was formalized in 2001 by LeRoy and Medsger with the publication of criteria for the classification of early SSc [3]. Subsequent studies have validated these criteria, demonstrating that the vast majority of early SSc patients will develop definite SSc [2, 4, 5]. Nevertheless, a definite answer to the question of when is scleroderma really scleroderma? is still to come. A fundamental endeavour to tackle this issue has been undertaken by the ACR/European League Against Rheumatism (EULAR) Scleroderma Classification Criteria Committee.
which proposed new criteria to identify definite SSc patients [6]. The availability of these criteria allows us to reassess the genetic, demographic and clinical factors that influence the progression or rate of progression of the disease. Herein we considered a prospective case series of early SSc patients to test the hypothesis that the time to definite SSc may be related to class II HLA—a well-characterized genetic risk factor of SSc [7, 8]—or to other factors associated with disease progression, including NVC [2, 4, 5] and SSc-specific autoantibodies [2, 5].

**Methods**

One-hundred- and fifty-eight consecutive adult subjects with RP fulfilling the Leroy and Medsger criteria for early SSc [3] (e.g. RP plus the presence of either SSc-specific autoantibodies and/or SSc-specific NVC alterations) without any manifestation indicative of definite SSc [6] who were referred to our centre between 1998 and 2010 were considered. In our centre, patients are periodically evaluated with complete clinical examination, pulmonary function testing and echocardiography; the suspicion of pulmonary arterial hypertension (PAH) and interstitial lung disease (ILD) is then confirmed via right heart catheterization and high-resolution chest tomography, respectively. This ensured that all the patients met the eligibility criteria and allows an accurate retrospective reconstruction of the time to the event, i.e. the time from referral to the development of definite SSc according to van den Hoogen et al. [6]: patients with a score ≥ 9 are classified as definite SSc, where the single-item scores are skin thickening of the fingers of both hands extending proximal to the MCP joints (score = 9); the highest score between puffy fingers (score = 2) and skin thickening of the fingers (score = 4); highest score between digital tip ulcers (score = 2) and pitting scars (score = 3); telangiectasia (score = 2); abnormal nailfold capillaries (score = 2); lung involvement (ILD/PAH; score = 2); RP (score = 3) and SSc-specific antibodies (score = 3).

The following baseline characteristics were considered: age at the onset of RP, delay from RP onset and referral, autoantibody subset and NVC pattern assessed by an experienced observer (M.C.) and classified according to Cutolo et al. [9]. ANAs were determined by indirect immunofluorescence on Hep2 cells (Kallestad, Chaska, MN, USA) [10]; ENAs, including anti-topoisomerase I (Scl70), ACA, anti-PM-Scl, anti-RNA polymerase I or III and anti-fibrillarin antibodies, were determined by a commercial ELISA (Diamedix, Miami, FL, USA).

High-resolution HLA class II typing was performed by means of PCR with sequence-specific primers as previously described [11]. HLA haplotypes were reconstructed via Phase software [12] (available at http://stephenslab.uchicago.edu/software.html).

All subjects gave their written consent to have their genetic, laboratory and epidemiological data anonymously used for this study, which was approved by the local ethics committee (comitato etico Area B Milano).

Time-to-event analysis up to 10 years was applied to baseline genetic and demographic variables; all the patients had at least 12 months of observation from baseline. Kaplan–Meier curves were drawn to estimate survival times, which were compared by means of the log-rank test. Cox regression analysis was used to estimate the hazard ratios (HRs) along with their 95% CIs. Underrepresented HLA alleles and haplotypes with a frequency <5% were excluded; 10 000-fold permutation testing was used to correct log-rank test values and to circumvent the inflation of type I error due to multiple comparisons while preserving the dependence structure among HLA alleles [13]. Statistics were performed using SPSS 20.0 software (IBM, Armonk, NY, USA).

**Results**

Baseline demographic and clinical characteristics of the 158 early SSc patients are reported in supplementary Table S1, available at *Rheumatology* Online. SSc-specific antibodies were present in 140 patients (88.6%), abnormal nailfold capillaries were present in 151 (95.6%); both SSc-specific antibodies and abnormal nailfold findings were observed in 133 (84.2%) at baseline, therefore 25 patients (15.8%) fulfilled just one extra-RP criterion for the diagnosis of early SSc (18 fulfilled the autoantibody criterion and 7 the capillaroscopy criterion). In our dataset, right censorship was equal to 31% and the median estimated survival time was 45 months from referral (95% CI 30.6, 59.4). Estimates of progression at 5 and 10 years were 59.8% and 80%, respectively, with a crude number of progressors equal to 109 cases. Puffy fingers were the most frequent evolution feature, alone or in combination with other clinical items (n = 89 cases, 81.7%). Supplementary Table S2, available at *Rheumatology* Online, lists in detail the evolution features in our population of early SSc patients.

Patients with ACA had a reduced risk of progression compared with those not bearing this antibody [median 55 vs 23 months, P = 0.026, HR 0.655 (95% CI 0.448, 0.956)]. Patients with the active/late NVC pattern had shorter times to definite SSc compared with those with a slow capillaroscopic pattern [median 24 vs 55 months, P = 0.041, HR 1.478 (95% CI 1.010, 2.163)]. The slow NVC pattern was associated with the presence of ACA (Pearson’s chi squared 9.154, P = 0.002) and when the analysis was restricted to patients satisfying both anticlonal and NVC criteria for the diagnosis of early SSc (n = 133), the predictive effect of the capillaroscopic pattern was dampened (P = ns), suggesting that its role in disease progression is dependent on the concurrent presence of ACA. Duration of RP, gender, age at referral or age at onset of RP were not relevant to disease progression.

Supplementary Table S3, available at *Rheumatology* Online, summarizes the time-to-event analysis for the alleles and haplotypes that passed the initial quality control and for which the uncorrected log-rank test value was significant at the 0.05 threshold. The HLA-DRB1*0101, HLA-DQA1*0101(4) and HLA-DQB1*0501 alleles, located
within the DQ5-DR1 haplotype, conferred protection towards evolution to definite SSc. All the results were confirmed after correction for the presence of ACA or NVC pattern.

Table 1 reports the results of the survival analysis conducted at the genotype level. Here it can be seen that ACA and the DQ5-DR1 genotype exert an additive effect on disease progression: ACA-positive subjects carrying one or two copies of the DQ5-DR1 haplotype (DQ5-DR1/x, DQ5-DR1/DQ5-DR1) have a decreased risk of progression compared with ACA-negative patients not bearing any copy of the DQ5-DR1 allele (x/x genotype) or ACA-positive subjects with the x/x genotype (Fig. 1); DQ5-DR1 carriers negative for ACA (n = 6) were excluded from the analysis due to unreliable survival estimates in this small group of patients. ACA positivity was evenly distributed in DQ5-DR1/x and/or DQ5-DR1/DQ5-DR1 patients (Pearson’s chi squared or Fischer’s exact test, P = ns), suggesting independence between these variables and underlying the validity of the interaction model in the absence of multicolinearity [14]. Scl70 or RNA Pol antibodies had no influence on the time to event (log-rank test P = 0.069 and P = 0.099, respectively), yet a small interaction effect with the DQ5-DR1 haplotype could be observed due to the fact that Scl70-negative or RNA Pol-negative patients are mostly ACA positive (see supplementary Table S4, available at Rheumatology Online).

All the genetic results, both at allelic and genotypic levels, were confirmed when the analysis was restricted to patients satisfying both anticorpal and NVC criteria for the diagnosis of early SSc.

**Table 1** Time-to-event analysis at the genotype level

<table>
<thead>
<tr>
<th>HLA</th>
<th>Evo, n (%)</th>
<th>No Evo, n (%)</th>
<th>HR (95% CI)</th>
<th>Survival (95% CI)</th>
<th>LRp</th>
<th>Perm P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*0101</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>99 (73.3)</td>
<td>36 (26.7)</td>
<td>1 (reference)</td>
<td>43 (25.6, 60.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>10 (43.5)</td>
<td>13 (56.5)</td>
<td>0.39 (0.2, 0.75)</td>
<td>108 (NA)</td>
<td>0.003</td>
<td>0.041</td>
</tr>
<tr>
<td>DRB1*0103</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>97 (66.9)</td>
<td>48 (33.1)</td>
<td>1 (reference)</td>
<td>48 (32.1, 63.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>12 (92.3)</td>
<td>1 (7.7)</td>
<td>1.88 (1.03, 3.43)</td>
<td>20 (5.9, 34.1)</td>
<td>0.035</td>
<td>NS</td>
</tr>
<tr>
<td>DQA1*0101</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>92 (73.6)</td>
<td>33 (26.4)</td>
<td>1 (reference)</td>
<td>43 (27.2, 58.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>17 (61.5)</td>
<td>16 (38.5)</td>
<td>0.6 (0.34, 0.93)</td>
<td>106 (60.9, 151)</td>
<td>0.022</td>
<td>NS</td>
</tr>
<tr>
<td>DQB1*0501</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>91 (74)</td>
<td>32 (26)</td>
<td>1 (reference)</td>
<td>39 (22.5, 55.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>18 (51.4)</td>
<td>17 (48.6)</td>
<td>0.51 (0.31, 0.86)</td>
<td>106 (41.6, 170.3)</td>
<td>0.008</td>
<td>0.049</td>
</tr>
</tbody>
</table>

**Interactions**

| ACA− and DQ5-DR1− | 46 (79.3) | 12 (20.7) | 1 (reference) | 22 (16.8, 27.2) | 0.15   | NA     |
| ACA− and DQ5-DR1+ | 54 (69.2) | 24 (30.8) | 0.75 (0.5, 0.11)| 47 (37.2, 56.7) |         |        |
| ACA+ and DQ5-DR1+ | 5 (31.2)  | 11 (68.8) | 0.22 (0.09, 0.57)| >120 (NA)      | 0.002   | NA     |

**LRp** = 0.01, **HR** = 0.3 (95% CI 0.12, 0.752) for the pairwise comparison ACA− and DQ5-DR1− vs ACA+ and DQ5-DR1+. ACA− and DQ5-DR1− individuals (n = 6) are not reported due to unreliable survival, HR and 95% CI estimates. Evo: evolution from early [3] to definite systemic sclerosis [6]; HR: hazard ratio by Cox regression; LRp: log-rank test P-value; NA: not applicable; NS: not significant; Perm P: 10000-fold permutation test P-value; Survival: estimated survival time by the Kaplan-Meier method.

**Discussion**

The study of systemic autoimmune diseases in their earliest phases is a field of great interest that may help to stratify patients for prognostic purposes and help us understand the biological mechanisms that determine the progression of these disorders. The precise definition of both early (preclinical) and definite disease is mandatory to reduce the bias in the collection of data and to provide reproducible and homogeneous results across different populations where the same criteria are applied. While the definition of early SSc has long been formalized [3] and its prognostic significance verified [2], only in 2013 did the ACR and EULAR jointly provide new SSc classification criteria that may allow the early identification of definite SSc patients with improved sensitivity and specificity compared with the criteria that are commonly used [6]. When we undertook our work only one report described the course of the disease in a case series (n = 60) of early SSc applying the 2013 ACR/EULAR criteria [6]. Several considerations can be drawn from our data.

First, we showed that the vast majority of early SSc patients will progress towards definite SSc within 5–10 years of their diagnosis. Our case series of early SSc patients had subjects positive for SSc-specific antibodies, and this may explain the high rates of progression we observed. The occurrence of autoantibodies was previously identified as a predictor of progression to definite SSc by Koenig et al. [2] and Valentini et al. [5], who concluded that SSc-specific autoantibodies are a risk factor.
for shorter survival times in early SSc patients. The latter authors did not report median survival as calculated by the product-limit estimator, preventing a direct comparison with our data. However, applying the method of Parmar et al. [15] to figure 1 of Valentini et al. [5], a median time to progression of between 38 and 48 months can be extrapolated, which is largely comparable to the value we observed.

Second, we showed that the antibody subset is relevant to the time of progression, with longer survival times associated with ACA. These results are at odds with Valentini et al. [5]; however, their study was underpowered to detect meaningful differences among autoantibody specificities. In the preliminary analysis we conducted, different capillaroscopy pictures were associated with different rates of progression, most likely as a consequence of the correlation between the slow NCV pattern and ACA, as described elsewhere [16].

Lastly, we showed that HLA class II alleles influence the progression from early to definite SSc and that the DQ5-DR1 haplotype strongly reduces the risk of progression and lengthens the time to evolution independently of the presence of ACAs or other baseline characteristics. The protective effect of the DQ5-DR1 haplotype was found to be additive to ACAs in DQ5-DR1/x and DQ5-DR1/DQ5-DR1 individuals. Other studies have demonstrated that antigens within the DQ5-DR1 haplotype may influence disease severity in SSc and other systemic autoimmune diseases. HLA-DRB1*01 was found to be negatively correlated with the occurrence of ILD in Brazilian SSc patients [17]. Moreover, protection against all manifestations of sarcoidosis was observed in those bearing HLA-DRB1*01 or HLA-DQB1*05 antigen [18]. The precise mechanism by which HLA antigens modify the time to evolution of the disease is unknown and it may be speculated that antigen presentation by HLA specificity may affect immune function, but further studies are needed to clarify these aspects.

To better gauge the implications of our study its shortcomings should be acknowledged. At the moment it is not possible to determine whether our findings can be extended to other populations of early SSc patients. The phenotypic expression of HLA class II alleles in complex diseases is largely dependent on the genetic background of affected individuals [19], which greatly changes across different geographical regions [20]. Indeed, previous reports have demonstrated that the effect of HLA alleles on SSC susceptibility or clinical expression is significantly different in populations with uncommon ancestry [7, 8]. Moreover, our cases series was composed of a selection of early SSc patients referred to a tertiary centre, which may not reflect the wider population of early SSc patients. The vast majority of our early SSc patients had both abnormal NVC findings and SSc-specific antibodies, while only a small proportion of them had just one of two extra-RP criteria for early SSc (15.8%) [3]. It has been suggested that early SSc patients without antibodies have a reduced risk of progression towards definite SSc according to the 2013 ACR/EULAR criteria [5] or according to more general clinical criteria [2, 4], hence caution should be exercised in the extension of our findings to this subset of patients.

In summary, in our work we characterized the natural history of early SSc in a consistent case series of Italian patients, describing the factors that influence progression towards definite SSc and highlighting the importance of autoantibodies, HLA class II alleles and their interaction. The consequences of these findings in terms of prognostication or in gaining a better understanding of SSc pathophysiology are still to be defined.

**Rheumatology key messages**

- The vast majority of early SSc patients progress towards definite SSc.
- Autoantibody specificities are associated with different times to definite SSc.
- HLA alleles within the DQ5-DR1 haplotype influence the time to definite SSc.

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**Disclosure statement:** The authors have declared no conflicts of interest.
Supplementary data

Supplementary data are available at Rheumatology Online.

References