HLA allelic variants encoding DR11 in diffuse and limited systemic sclerosis in Caucasian women

L. S. Loubière1, N. C. Lambert1,6, M. M. Madeleine4, A. J. Porter1, M. E. Mullarkey1, J. M. Pang1, D. A. Galloway2, D. E. Furst5 and J. L. Nelson1,3

Objective. We investigated HLA class II alleles in women with systemic sclerosis (SSc), a rare disease that preferentially affects women.

Methods. Specific alleles of DRB1, DQA1 and DQB1 were determined by DNA-based HLA typing for women with SSc \( (n = 102) \) and healthy women \( (n = 533) \). All study subjects were Caucasian. DRB1, DQA1 and DQB1 allele frequencies of women with SSc were compared with those of healthy women.

Results. Among women with SSc, 29.4% (30/102) and among healthy women 10.7% (57/533) had DRB1*11. Allele frequencies were compared for women with SSc and healthy women (each woman has two alleles). The allele frequency of DRB1*11 was 15.7% (32/204 alleles) in SSc women and 5.8% (62/1066 alleles) in healthy women \( (P = 0.000002) \). The increase of DRB1*11 was found both in diffuse \( (P = 0.0001) \) and limited SSc \( (P = 0.002) \) (allele frequencies 15.0 and 17.2%, respectively). Among women with diffuse SSc, there was a disproportionate increase of the DRB1*1104 allele \( (P = 0.0004) \) with no increase of DRB1*1101 \( (P = 1.00) \). In contrast, in limited SSc the strongest association was with DRB1*1101 \( (P = 0.008) \), with a less significant increase of DRB1*1104 \( (P = 0.04) \).

Conclusions. An increase of DRB1*11 in SSc is consistent with other reports. Although present in both diffuse and limited SSc disease subsets, the increase was predominantly due to over-representation of DRB1*1104 in women with diffuse SSc. Women with limited SSc had a preponderance of DRB1*1101, the most common allele in healthy women. DRB1*1104 and DRB1*1101 differ by a single amino acid at position 86, where the former has valine and the latter glycine.

Key words: Systemic sclerosis, HLA alleles, HLA-DRB1, Gender.

Systemic sclerosis (SSc) or scleroderma has a predilection for women and particular HLA class II molecules are associated with increased disease risk. Thus, both gender and genotype contribute to SSc susceptibility.

Among Caucasians, the most consistently described HLA association in SSc has been DRB1*11 [1] with DRB1*03 sometimes reported as increased [2] and occasionally DRB1*01 [3]. In addition to differences in HLA associations according to race/ethnic origin, differences have been reported when patients are considered according to disease subset and when considered according to specific SSc-associated antibodies (reviewed in [4]). Most reports describe patient and control populations that combine women and men. However, two studies that provided analyses stratified by gender suggested differences in the HLA associations of men and women with SSc [5, 6]. We initiated the present study for several reasons. First, reports have been variable as to the specific DRB1*11 allele associations in SSc and few studies have evaluated DRB1*11 alleles according to the clinically defined disease subsets, diffuse or limited SSc. Additionally, long-term persistence of fetal cells from pregnancy has recently been implicated in SSc [7], so that HLA-associated risk could differ in women and men, as has also been suggested in other studies [5, 6]. We therefore determined HLA allele associations in a cohort exclusively of women, conducting DNA-based typing to identify specific allelic variants of DRB1, DQA1 and DQB1.

Patients and methods

Subjects

A total of 635 unrelated subjects were studied. All subjects were Caucasian. Study subjects included 102 women with SSc and 533 healthy women. Because SSc is a rare disease [8] patient recruitment included all patients referred from the practices of rheumatologists in metropolitan Seattle and Washington State, although some patients had their primary residence in states such as Montana, Oregon and Alaska and a few were self-referred from other states. Control healthy women were recruited locally either by flyers requesting volunteers \( (n = 152) \); control group A or as healthy controls as part of other studies \( (n = 381) \); control group B) as previously described [9]. Subjects with SSc were classified as having diffuse SSc (dcSSc; \( n = 70 \)) or limited SSc (lcSSc; \( n = 32 \)) according to the diagnosis of the treating rheumatologist. The mean age at disease onset was 42 yr (range 15–70) for patients.

1Immunogenetics Program and 2Cancer Biology, Fred Hutchinson Cancer Research Center, Seattle, WA, 3Division of Rheumatology, University of Washington, Seattle, WA, 4Program in Epidemiology, Fred Hutchinson Cancer Research Center and Department of Epidemiology, University of Washington, Seattle, WA and 5Rheumatology, University of California, Los Angeles, CA, USA.

Present address: Laboratoire INSERM, U639, Immunogenetics of Rheumatoid Arthritis, Faculté de médecine de la Timone, Marseille, France.

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Correspondence to: L. S. Loubière, Immunogenetics Program, D2-100, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, Seattle, WA 98109, USA. E-mail: lloubiere@fhcrc.org

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with dcSSc and 46 yr (range 25–73) for patients with lcSSc. All subjects provided informed consent.

DNA extraction

DNA was extracted from either whole heparinized blood or peripheral blood mononuclear cells that had been isolated by Ficoll Hypaque (Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation at 1.077 g/ml, using an Isoquick Nucleic Acid Extraction Kit (Orca Research, Bothell, WA, USA) according to manufacturer’s instructions and resuspended in Tris–HCl (10 mM, pH 9.0). For some study subjects, DNA was extracted from mouthwash specimens according to methods described by Lum and Le Marchand [10] with DNA isolation using a high pure PCR template preparation kit (Boehringer Mannheim, Indianapolis, IN, USA) and resuspended in Tris–HCl (10 mM, pH 9.0).

HLA genotyping

DNA-based HLA typing was conducted to determine specific DRB1, DQA1 and DQB1 alleles. DQA1 and DQB1 alleles were determined by sequence-specific oligonucleotide probe (SSOP) typing with methods similar to those previously described [11], to which other probes were added to detect newly identified alleles of DQA1 and DQB1 [12]. For DRB1, a low-resolution assay was used that detects the DRB1 families DRB1*01 to DRB1*14, followed by one or more high-resolution assays that identify specific DRB1 alleles as previously described [7]. Alternatively, some DRB1 low-resolution assays and DQB1 typing was done using a Dynal Reli™ SSO HLA-DRB or DQB typing kit (Dynal, Oslo, Norway), following the manufacturer’s instructions with subsequent direct sequencing to determine the specific allele. At least one family member was also HLA- genotyped for the majority of the women with SSc (79.4%) and healthy women of control group A (78.3%) in our study, so that specific HLA DRB1-DQA1-DQB1 haplotypes could be assigned.

Statistical analysis

Allele frequencies of Caucasian women with SSc were compared with allele frequencies of healthy Caucasian women. For each group, allele frequencies were calculated by counting the number of DRB1 alleles, for example for DRB1*01, and dividing by the total number of alleles (two times the number of individuals in the group, since each person has two DRB1 alleles). Comparisons were made using the χ² test for 2 × 2 contingency tables with Yates-corrected P values, when all expected cell values were ≥5, or the two-tailed Fisher’s exact test P value, when an expected cell value was ≤5. Exact methods were used to compute two-sided P values using the Epi-Info statistical program (Centers for Disease Control and Prevention, Atlanta, GA). Analyses were examined for DRB1, DQA1 and DQB1 allele families if present in at least 5% of cases or controls. For DRB1, DQA1 or DQB1 alleles this included eight DRB1 families (DRB1*01, *15, *03, *04, *11, *13, *07, *08), seven for DQA1 (DQA1*0101, *0102, *0103, *02, *0301, *04, *0501) and eight for DQB1 (DQB1*02, *0301, *0302, *04, *0501, *0602, *0603). Additional analysis was conducted for specific DRB1*11 alleles, including DRB1*1101, *1102, *1103, *1104 and *1111. We did not correct for multiple comparisons in this study as our purpose was to confirm and refine the previously reported DRB1*11–SSc association.

This study was approved by the Fred Hutchinson Cancer Research Center Human Subjects Review Committee.

Results

DRB1*11 was found among 29.4% (30/102) of women with SSc and 10.7% (57/533) of healthy women. Allele frequencies were very similar in the two control populations (group A and group B); therefore, they were combined for analysis. Allele frequencies (each individual has two alleles) were analysed for women with SSc compared with healthy women. We found that the allele frequency of DRB1*11 was significantly increased among women with SSc (15.7%) compared with healthy women (5.8%) (P = 0.000002) (Table 1). The DQB1*0301 allele is in linkage disequilibrium with DRB1*11, as demonstrated by HLA-typing of family members (see Patients and methods) and other reports [13, 14] and was increased to a lesser extent (24.5% vs 16.7%, P = 0.01). The DQA1*0501 allele is also in linkage disequilibrium with DRB1*11 and DQB1*0301, but was not significantly increased in women with SSc compared with controls (27.5% vs 23.9%, P = 0.33).

The allele frequency of DRB1*03 did not differ in women with SSc compared with healthy women (12.7% vs 14.5%, P = 0.60). Similarly, DRB1*07 was not increased among women with SSc compared with controls (10.3% vs 10.5%, P = 0.97). DRB1*07, previously described as a protective allele, was found with similar frequency in women with SSc and healthy women (11.3% vs 12.3%, P = 0.77). DRB1*07 is in linkage disequilibrium with DQA1*02 and DQB1*0303 or DQB1*0202 [13, 14], and was confirmed in

<p>| Table 1. HLA-DRB1 allele frequencies (%) in women with SSc and healthy women |</p>
<table>
<thead>
<tr>
<th>All SSc (n = 102)a</th>
<th>Diffuse SSc (n = 70)b</th>
<th>Limited SSc (n = 32)c</th>
<th>All controls (n = 533)d</th>
</tr>
</thead>
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<tr>
<td>Alleles (N = 204)</td>
<td>Alleles (N = 140)</td>
<td>Alleles (N = 64)</td>
<td>Alleles (N = 1066)</td>
</tr>
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<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>DRB1*01</td>
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<td>14</td>
</tr>
<tr>
<td>DRB1*03</td>
<td>26</td>
<td>12.7</td>
<td>18</td>
</tr>
<tr>
<td>DRB1*11</td>
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<td>15.7</td>
<td>21</td>
</tr>
<tr>
<td>DRB1*07</td>
<td>23</td>
<td>11.3</td>
<td>18</td>
</tr>
<tr>
<td>DQA1*0501</td>
<td>56</td>
<td>27.5</td>
<td>37</td>
</tr>
<tr>
<td>DQB1*0301</td>
<td>50</td>
<td>24.5</td>
<td>34</td>
</tr>
</tbody>
</table>

All study subjects were Caucasian.

'a' All controls' are controls consisting of all healthy women (see Patients and methods).

'n' number of patients in each group; Statistically significant P values (P < 0.05) are in bold. N, number of alleles in each group.

'DQA1 not available for five women with SSc and three for DQB1.'

'DQA1 not available for three women with deSSc and two for DQB1.'

'DQA1 not available for two women with lcSSc and one woman for DQB1.'

'DQA1 not available for seven healthy women and three for DQB1.'
In the present study we examined HLA class II allele frequencies in women, comparing SSc women with healthy women in a Caucasian population. The significant increase of DRB1*11 that we observed is confirmatory of prior studies in other Caucasian populations that included both men and women [1, 3, 15–17] and consistent with another USA population studied in which DRB1*11 was increased, although not significantly [18]. When analysed according to the clinically defined SSc subsets, DRB1*11 was significantly increased both in dcSSc and lcSSc. These results also confirm other reports of increased DRB1*11 frequency in diffuse disease [1, 15–16] and limited disease [3, 15, 17].

We initiated the present study to examine allelic variants of DRB1*11 in a cohort limited to women. We found differences in diffuse and limited subsets in allelic variants within the DRB1*11 family. Among women with dcSSc the DRB1*11B4 allele was significantly increased. The most common DRB1*11 allele in healthy individuals was DRB1*1101 and this allele was further increased in lcSSc, with less of an increase of DRB1*1104, although our ability to draw conclusions for this subset is limited because our study included fewer women in this category. A few other studies have examined allelic variants of DRB1*11 with variable results. Considering our results along with prior studies lends support to the conclusion that the primary association of dcSSc is with the DRB1*1104 allele. In one study of Greeks, an increase of DRB1*1104 in dcSSc but not in lcSSc or overlap was reported [19], and in another small study of Mexican Mestizos DRB1*1104 was primarily found in dcSSc [20]. Other studies that considered SSc patients categorized according to whether they had antibodies to topoisomerase described variable results: most reported an increase of DRB1*1104 [21–23] and others an increase of DRB1*1101 [18] or DRB1*1101 and 1104 [24].

We found an equal prevalence of DRB1*03 in women with dcSSc compared with healthy women. Whether or not DRB1*03 is increased in SSc has been controversial, some reports describing an increase in diffuse disease [16, 17] while others have not [18, 19, 24]. However, most studies did not stratify by gender, and our results, when considered along with two prior studies that did stratify by gender, suggest that this association differs depending on gender [5, 6]. In a study conducted prior to the development of DNA-based HLA typing techniques, Lunderschmidt et al. reported an increase of DR3, the molecule encoded by DRB1*03, that was only observed when men were analysed [5]. In our prior USA–German collaborative study of SSc in men we also found an increase of DRB1*03, although the studied population was smaller [6]. Potential modulation of HLA-associated risk by gender is further underscored by recent findings of fetal cells that persist for decades after pregnancy (referred to as microchimerism) and may be implicated in autoimmune diseases such as SSc [25]. Fetal microchimerism could potentially impact disease risk in women our study by segregation in families (see Patients and methods). Interestingly, compared with healthy women, the DRB1*07-DQA1*02-DQB1*0303 haplotype was more frequent in women with SSc: 6.1% in women with SSc compared with 2.8% in the healthy women (12/198 vs 30/1060) (P = 0.04). The DRB1*07-DQA1*02-DQB1*0202 haplotype was less frequent in women with SSc than healthy women: 5.6 vs 9.2% (11/198 vs 98/1060, P = 0.12).

In further analysis, HLA allele frequency was considered after categorizing the 102 women according to whether the woman had dcSSc (n = 79, 69%) or lcSSc (n = 32, 31%). The allele frequency of DRB1*11 was significantly increased in dcSSc (15%) and in lcSSc (17.2%) compared with healthy women (5.8%) (P = 0.0001 and P = 0.002, respectively) (Table 1). The DQB1*0301 allele was increased in women with dcSSc (24.3%) compared with healthy women (16.7%) (P = 0.04) and in women with lcSSc (25.0%) compared with healthy women (16.7%) (P = 0.12) (Table 1).

The allele frequencies for specific DRB1*11 alleles in women with SSc and healthy women are summarized in Table 2. In healthy women and women with lcSSc, the most common DRB1*11 allele was DRB1*1101 (56.4 and 63.6%, respectively). In contrast, DRB1*1104 was predominant among women with dcSSc (52.3%). Among women with dcSSc, the frequency of the DRB1*1104 allele was significantly increased compared with healthy women, 7.9 vs 1.9% (P = 0.0004) and DRB1*1101 was not increased: 2.9 vs 3.3% (P = 1.00) (Table 2). In women with lcSSc, although DRB1*1104 was also increased compared with controls [6.3 vs 1.9% (P = 0.04)] the DRB1*1101 allele was more over-represented compared with controls: 10.9% vs 3.3% (P = 0.008).

Among women with dcSSc who carried DRB1*11 (n = 21), 11 (52%) had antibodies against topoisomerase I (Scl-70) and 10 (48%) did not. DRB1*1104 was more frequent in patients who were Scl-70-positive compared with Scl-70-negative patients, whereas DRB1*1101 was more frequent in patients who were Scl-70-negative. Among women positive for Scl-70, 73% (8/11) had DRB1*1104, none had DRB1*1101 and 27% (3/11) had DRB1*1103. Among women negative for antibodies to Scl-70, 33% had DRB1*1104 (3/10), 40% had DRB1*1101 (4/10) and the others had DRB1*1103 (2/10) or DRB1*1102 (1/10).

Discussion

In the present study we examined HLA class II allele frequencies in women, comparing SSc women with healthy women in a Caucasian population. The significant increase of DRB1*11 that

<table>
<thead>
<tr>
<th>HLA allele or allele group</th>
<th>All SSc (n = 102)</th>
<th>Diffuse SSc (n = 70)</th>
<th>Limited SSc (n = 32)</th>
<th>All controls (n = 533)</th>
<th>All SSc vs all controls</th>
<th>Diffuse SSc vs all controls</th>
<th>Limited SSc vs all controls</th>
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<tr>
<td>DRB1*11</td>
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<td>15.7</td>
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<td>15.0</td>
<td>11</td>
<td>17.2</td>
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<td>35</td>
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<tr>
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<tr>
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<td>5</td>
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<td>7.4</td>
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<td>7.9</td>
<td>4</td>
<td>6.3</td>
<td>20</td>
</tr>
<tr>
<td>DRB1*1111</td>
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<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
</tr>
</tbody>
</table>

All study subjects were Caucasian.

‘All controls’ are controls consisting of all healthy women (see Patients and methods).

n = number of patients in each group; N = number of alleles in each group.

Twenty-one women with dcSSc had one DRB1*11 allele; nine women with lcSSc had a DRB1*11 allele, and among them two had two DRB1*11 alleles (homozygous); 57 healthy controls had a DRB1*11 allele, and among them five had two DRB1*11 alleles (homozygous).
either to their benefit or detriment depending upon the specificity of the fetal (paternally contributed) HLA alleles.

The primary DQB1 and DQA1 alleles in linkage disequilibrium with DRBI*11 in Caucasians are DQB1*0301 and DQA1*0501, as previously reported [13, 14] and also observed in our study population, confirmed by segregation with at least one family member in the majority of our patients. A weaker association was observed with the DQB1 allele and no significant association with DQA1 in our study. In our prior study of SSC in men the predominant significant association was with DQA1*0501 without a significant increase of DRBI*11 [6]. However, we cannot make a direct comparison with the population studied in the present report since our prior report included cases and controls from Germany and the USA, because SSC is rare, and is all the more rare in men [8].

Our incidental finding of a differential distribution of DRBI*07 haplotypes in women with SSC compared with healthy women is of additional potential interest. Haplotypes with DRBI*07 include a DRB4 gene but those with DQB1*03 have been reported to not express the DRB4 cell surface product (DR53), in contrast to haplotypes with DQB1*02 [16, 27]. Sutton et al. showed that a DRB4 null allele is not expressed at the cell surface because of an altered splice site leading to an aberrant transcript [28]. The significance of the increased frequency of a DRB4 null allele in women with SSC is not clear, but others have speculated that antigenic peptides derived from degradation of products of the DRB4 null gene transcript could be presented to cells by other HLA molecules and lead to alloreactivity [28].

In summary, we found that DRBI*11 was strongly associated with dcSSc and lcSSc in Caucasian women. The distribution of DRBI*11 alleles, however, differed according to disease subset, with DRBI*1104 predominant in dcSSc and not in lcSSc, where DRBI*1101 was most common. DRBI*1101 and DRBI*1104 differ at position 86 of the β chain, where DRBI*1101 encodes a glycine and DRBI*1104 encodes a valine. Although the only difference between the two alleles is a single amino acid, functional studies have described a significant effect of position 86 on peptide binding [29], including naturally processed peptides [30] and autoantigen presentation to T cells [31].

Key messages

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- DRBI*11 was strongly associated with diffuse and limited SSC in Caucasian women.
- The distribution of DRBI*11 alleles differed according to disease subset: DRBI*1104 was predominant in diffuse SSC but not in limited SSC, where DRBI*1101 was more common.

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The authors have declared no conflicts of interest.

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