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HLA–DRB1, DQA1, and DQB1 in Juvenile-Onset Systemic Sclerosis

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Abstract

Objective.—Systemic sclerosis (SSc) is a rare disease that is particularly uncommon in children. Specific HLA alleles have been associated with SSc in adults. This study was undertaken to investigate HLA class II alleles in juvenile-onset SSc.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Stevens had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Methods.—DRB1, DQA1, and DQB1 alleles were determined by DNA-based HLA typing. Analyses were conducted comparing Caucasian patients with juvenile-onset SSc (n = 76) to healthy Caucasian controls (n = 581).

Results.—Initial analyses focused on HLA class II associations previously reported in adult Caucasian patients with SSc. The frequency of DRB1*11 was not significantly increased in juvenile-onset SSc (22.4% of patients with juvenile-onset SSc versus 17.6% of controls; odds ratio [OR] 1.35, $P = 0.34$), nor were the specific DRB1*11:01 or *11:04 alleles. DQA1*05, a risk factor previously identified in adult men with SSc, was increased in patients with juvenile-onset SSc versus controls (57.9% versus 44.1%; OR 1.76, $P = 0.027$), as was DRB1*03 (34.2% versus 22.5%; OR 1.79, $P = 0.031$). Secondary analyses of all DRB1 allele groups revealed an association with DRB1*10 (10.5% of patients with juvenile-onset SSc versus 1.5% of controls; OR 7.48, $P = 0.0002$). As this is a new observation, correction was made for multiple comparisons of 13 different DRB1 allele groups; results nevertheless remained significant ($P = 0.003$). Also, a lower frequency of DRB1*01 was observed in patients with juvenile-onset SSc who were younger at disease onset (OR 0.06, $P = 0.01$) and in those with antibodies to topoisomerase (OR 0.14, $P = 0.024$).

Conclusion.—Associations of HLA alleles with juvenile-onset SSc differed from associations with SSc in women, but were similar to associations with SSc in men. Additionally, a novel association with DRB1*10 was observed in children. The greatest proportion of genetic risk of SSc is contributed by the HLA complex, and the current study reveals the importance of the association of HLA class II genes in juvenile-onset SSc.

Systemic sclerosis (SSc) is a chronic inflammatory disease leading to end-organ fibrosis with significant morbidity and mortality. The etiology of SSc is unknown. The concordance rate in twins is low, but a genetic contribution has been demonstrated in gene association studies (1,2), with the HLA class II region being the most significantly associated region by far. Therefore it is likely that, as in other autoimmune diseases, HLA molecules contribute directly to the pathogenesis of SSc.

SSc is an uncommon autoimmune disease, and juvenile-onset SSc, defined as, <16 years of age at onset, is extremely rare, estimated at, <5% of all SSc patients (3,4). SSc in children shares features with adult SSc, but with notable differences (3). Unlike adult patients with SSc, those with juvenile-onset SSc have a greater predominance of diffuse cutaneous SSc (dcSSc) and overlap syndromes (dermatomyositis–scleroderma overlap). Patients with juvenile-onset SSc also have higher survival rates related to lower rates of renal crisis, symptomatic lung disease, and pulmonary hypertension (3–6). Although numerous studies have investigated HLA associations in adult SSc, age at onset has not generally been considered. Only 1 previous study has examined HLA alleles in juvenile-onset SSc, but all 27 patients in that study had overlap syndrome (7). To address the lack of studies including cohorts of patients with juvenile-onset SSc without overlap syndromes, we established a cooperative effort of 3 centers in the US with juvenile-onset SSc cohorts, to test the association of HLA–DRB1, DQA1, and DQB1 alleles previously reported in adult SSc with that in juvenile-onset SSc.

PATIENTS AND METHODS

Study subjects and HLA genotyping.

Patients were recruited between 1993 and 2014 at 3 centers. The Seattle center included patients from the Seattle Children's Hospital, the University of Washington, and the Fred Hutchinson Cancer Research Center, and additional patients whose data were contributed by collaborators in the Childhood Arthritis and Rheumatology Research Alliance (including the Mayo Clinic [Rochester, MN], the Lurie Children's Hospital, Hackensack University Medical Center, and the Lucile Packard Hospital [<http://www.caragroup.org>]). The other 2 centers were the Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center, and the Scleroderma Family Registry and DNA Repository based at the University of Texas Health Science Center at Houston. Human subjects committees approved the studies at each institution, and all research was carried out in compliance with the Declaration of Helsinki. Diagnoses were determined by rheumatologists at each institution. All included patients had disease onset at <16 years of age. Healthy Caucasian control subjects were recruited as part of other ongoing studies in Seattle and as part of other studies associated with the Scleroderma Family Registry in Houston. These control cohorts were combined in the analysis, as there were no significant differences in allele frequencies between sites. Study data were collected and managed using the Research Electronic Data Capture tool hosted at the University of Washington (<https://www.iths.org/investigators/services/bmi/redcap/>). HLA genotyping was conducted for the class II loci DRB1, DQA1, and DQB1 using sequence-specific oligonucleotide probe typing, Dynal Linestrips, or Luminox-based polymerase chain reaction–sequence-specific oligonucleotide probe techniques (One Lambda) (8,9).

Statistical analysis.

Statistical analysis was performed using GraphPad Prism 6 software. To compare data for patients with juvenile-onset SSc to that for controls, an exact *P* value was calculated using a 2-tailed Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using Woolf's method. For previously described associations (in adults), i.e., HLA-DRB1*01, *03, *07, *11 (*11:01 and *11:04), DRB1*15 (*15:01), DQA1*05, DQB1*02, *03 (*03:01), and DQB1*05 (*05:01), *P* values were presented without correction. Secondarily, all allele groups were examined for novel associations using Bonferroni correction for the number of comparisons. *P* values less than 0.05 were considered significant.

RESULTS

Clinical features.

Among the 85 patients with juvenile-onset SSc enrolled in this study, 76 were Caucasian, 5 African American, 1 Asian, 1 Native American, and 2 of mixed race. Because HLA allele frequencies vary in different racial/ethnic populations, and only 9 patients were non-Caucasian, analyses were conducted comparing Caucasian patients with juvenile-onset SSc to healthy Caucasian controls. Patient characteristics from the 3 centers were similar with the exception of an increased frequency of dcSSc contributed by the Seattle cohort, and

more patients with anticentromere antibodies (ACAs) from Pittsburgh (Table 1). Four patients with juvenile-onset SSc had myositis overlap, and 2 had mixed connective tissue disease. Although the classification system is not validated for children, we estimated from clinical records that 63% of the patients with juvenile-onset SSc had limited cutaneous SSc (lcSSc) and 37% dcSSc. The frequencies of anti-topoisomerase I (anti-topo I) and ACA in patients with juvenile-onset SSc were lower than in adult patients, consistent with previous reports (4–6,10). Surprisingly, anti-topo I antibodies were significantly more prevalent in younger juvenile patients, while ACAs were less prevalent (see Supplementary Figure 1, available on the *Arthritis & Rheumatology* web site at <http://onlinelibrary.wiley.com/doi/10.1002/art.39765/abstract>).

Comparison to previously reported HLA associations with SSc in adults.

Alleles and allele groups that had been reported to be associated with SSc in adult patients were examined first. The frequency of HLA-DRB1*11, the allele group most consistently described in association with SSc in Caucasian adults (8,11–14), was not significantly increased in patients with juvenile-onset SSc compared to controls (22.4% versus 17.6%; OR 1.35, $P = 0.34$), nor were the frequencies of specific DRB1*11:01 or *11:04 alleles (Figure 1 and Supplementary Table 1, available at <http://onlinelibrary.wiley.com/doi/10.1002/art.39765/abstract>).

DQA1*05, which is in linkage disequilibrium with DRB1*11, DRB1*03, and some alleles of DRB1*13 and *14, has also been described as being increased in adult SSc (11,15). In the current cohort, the frequency of DQA1*05 was significantly increased, present in 57.9% of patients with juvenile-onset SSc compared to 44.1% of controls (OR 1.76 [95% CI 1.08–2.8], $P = 0.027$).

The frequency of DRB1*03, which was increased in some studies of adult SSc (16), was increased to an extent similar to the frequency of DQA1*05, present in 34.2% of patients versus 22.5% of controls (OR 1.79 [95% CI 1.07–2.98], $P = 0.031$). The frequency of DRB1*01, which has been associated with adult lcSSc and ACA positivity, was not increased in juvenile-onset SSc overall (17.1% of patients versus 23.8% of controls).

DRB1*07 has been described as protective for SSc in Caucasians (11,12). Although there was a trend toward a decreased frequency of DRB1*07 in patients with juvenile-onset SSc, the difference compared with controls was not significant (19.7% versus 27.9%). DRB1*07 is in linkage disequilibrium with DQB1*02:02 as well as DQB1*03:03. In contrast to studies of adult SSc in which the frequency of DQB1*02:02 was decreased (11), frequencies of DQB1*02:02 and DQB1*03:03 were not significantly different in juvenile-onset SSc versus controls.

Some studies have demonstrated a decreased frequency of DRB1*15 in Caucasians with SSc (11), while others have shown an increase (17). In patients with juvenile-onset SSc, there was a trend toward a decreased frequency of DRB1*15 compared to controls (17.1% versus 28.1%, respectively), but the difference was not significant. Comparing only patients in the lcSSc disease subset to controls, the OR for DRB1*15 was 0.44 ([95% CI 0.19–1.00], $P = 0.043$). A marginally significant decrease in the frequency of DQB1*06 was observed in

juvenile-onset SSc (OR 0.59 [95% CI 0.35–0.98], $P = 0.047$), mostly attributable to DRB1*06:02, which is in linkage disequilibrium with DRB1*15 (Figure 1).

Novel HLA associations in juvenile-onset SSc.

After considering the HLA alleles and allele groups according to previously reported associations in adults, our analysis of juvenile-onset SSc was extended to consider any DRB1, DQA1, or DQB1 associations that might be unique to juvenile-onset disease (Figure 1). A novel association was observed with DRB1*10, present in 10.5% of patients with juvenile-onset SSc compared to 1.5% of controls (OR 7.48 [95% CI 2.79–20], $P = 0.0002$). As this is a new observation, we conservatively corrected for 13 different DRB1 allele groups tested. The difference remained significant ($P = 0.003$).

Clinical features of juvenile-onset SSc and HLA associations.

Although the frequency of DRB1*01 was not significantly different between the total juvenile-onset SSc cohort and the healthy controls (see Supplementary Table 1, available at <http://onlinelibrary.wiley.com/doi/10.1002/art.39765/abstract>), there were several notable clinical differences among the patients with juvenile-onset SSc who were positive for DRB1*01. The frequency of DRB1*01 differed according to age at disease onset and autoantibody status (Figure 2). DRB1*01 was less prevalent in patients who were younger at disease onset (<6 years) compared to those who were older at disease onset (11–16 years) (OR 0.06 [95% CI 0.004–1.16], $P = 0.01$).

There was a close association between specific autoantibodies and disease type in juvenile-onset SSc; patients with anti-topo I were more likely to have dcSSc while those with ACA were more likely to have lcSSc. In studies of adult SSc, HLA alleles have been found to be associated with specific autoantibodies more closely than with clinical phenotypes (14). For instance, DRB1*01 was associated with ACA, which is more common in patients with lcSSc (18). This association was also supported in the current study; DRB1*01 was present in a significantly higher proportion of patients with juvenile-onset SSc who were ACA positive compared to patients who were anti-topo I positive (Figure 2). Frequencies of DRB1*01 did not differ significantly between patients with juvenile-onset SSc who were ACA positive and controls (38.5% versus 23.8%), although only a small number of patients were positive for ACA. However, frequencies of DRB1*01 were significantly decreased in patients positive for anti-topo I compared to controls (4.2% versus 23.8%; OR 0.14 [95% CI 0.02–1.04], $P = 0.024$). The frequency of DRB1*01 was also decreased in patients with dcSSc compared to controls (7.1% versus 23.8%; OR 0.25 [95% CI 0.06–1.05], $P = 0.04$). The difference may be at least partially attributed to the increased proportion of dcSSc positivity and for anti-topo I in the youngest patients, as DRB1*01 was less prevalent in patients who were younger at disease onset (Figure 2 and Supplementary Figure 1, available at <http://onlinelibrary.wiley.com/doi/10.1002/art.39765/abstract>).

The strong association of DRB1*10 with juvenile-onset SSc was present regardless of clinical phenotype. It was increased among both patients with dcSSc and those with lcSSc compared to controls (OR 10.6 [95% CI 3.04–36.86], $P = 0.002$ and OR 5.8 [95% CI 1.71–19.52], $P = 0.013$, respectively), as well as among patients who were positive for anti-topo I

(OR 9.1 [95% CI 2.29–36.00], $P = 0.01$), and there was a non-significant trend toward an increase among patients who were positive for ACA (OR 5.3 [95% CI 0.62–45.2], $P = 0.2$). No DQB1 allele was significantly associated with anti-topo I or ACA.

Specific amino acid sequences common to multiple HLA alleles have been considered in the effort to identify functionally important domains of the HLA molecules associated with SSc. The amino acid motif ⁷¹TRAEDLT⁷⁷ on the DQβ1 molecule, reported in association with anti-topo I positivity in SSc in some Caucasian populations (14,17,19), was not increased among our patients with juvenile-onset SSc overall or in the dcSSc or lcSSc subsets. The DRβ1 amino acid sequence ⁶⁷FLEDR⁷¹, described in 1 report as being increased in Caucasian patients with SSc (17), was not significantly increased in our patients with juvenile-onset SSc, although trends were essentially similar to what has been reported in adult SSc.

DISCUSSION

Although numerous studies of adult SSc have established the important role of the HLA class II region in genetic risk for SSc, only 1 has included juvenile patients, all of whom had dermatomyositis-scleroderma overlap (7). In this report, we describe the frequency of HLA-DRB1, DQA1, and DQB1 alleles in Caucasian patients with juvenile-onset SSc, recruited from 3 pediatric scleroderma centers.

We first sought to determine whether, in Caucasian populations, patients with juvenile-onset SSc have HLA allele associations similar to those identified in adult patients with SSc. The most consistent and most frequently observed association across multiple different Caucasian populations of adult SSc is with DRB1*11, especially the DRB1*11:04 and *11:01 alleles (8,11,13,14). Some studies have shown an increase in frequency of DRB1*03 and others have demonstrated an increased frequency of DRB1*01, the latter primarily in lcSSc (16,18). The allele groups DRB1*07, and to a lesser extent DRB1*15, have been described as protective, or negatively correlated with disease (11,12). At the DQA1 locus, DQA1*05 has been associated with SSc risk, especially in male subjects (11,15). At DQB1, the *03:01 allele has been associated with risk, DQB1*02 and *06:02 with protection, and DQB1*05:01 variably with risk or protection, depending on the disease subtype or particular SSc autoantibody (18).

The first notable observation in our study of juvenile-onset SSc was that the frequency of the DRB1*11 alleles were not significantly increased. This is in contrast with multiple prior studies in adults as described above, and including a study of SSc in adult women in which DRB1*11:01 and *11:04 were significantly increased (8). Interestingly, the frequency of DQA1*05 was significantly increased in patients with juvenile-onset SSc. DQA1*05 is in linkage disequilibrium with DRB1*11 as well as with DRB1*03 and some other less common DRB1 alleles. This result regarding allele association in juvenile-onset SSc is similar to results of a prior study of adult men with SSc, in which DQA1*05 was significantly associated with risk whereas DRB1*11 was not (15). A similar observation of increased frequency of DQA1*05 in men has since been reported in another study (11). Thus, the genetic features of juvenile-onset SSc observed in the present study resembled

those in adult male patients with SSc and differed from those in adult female patients with SSc in this respect. The frequency of DRB1*03 was also significantly increased in juvenile-onset SSc, but as it is in linkage disequilibrium with DQA1*05, it is not possible to determine whether one or the other locus is the primary association.

We further report a new observation. In our study population, the association of juvenile-onset SSc with DRB1*10 was the strongest and was evident in the different clinical subsets. To our knowledge this is the first description of an association of DRB1*10 with disease in an SSc population overall, as well as in a Caucasian population. It is noteworthy that in a study of a Chinese population, the frequency of DRB1*10 was increased in a secondary analysis restricted to ACA-positive patients (20).

The frequency of DRB1*07 and *15 allele groups was reduced in the patients with juvenile-onset SSc, suggesting a protective effect, although results were not significant for either allele group. This is consistent with findings in adults, in whom DRB1*07 is protective as described above, and in most studies of Caucasian populations (which also suggest the protective effect of DRB1*15). The increased frequency of DQA1*05 and DRB1*03 and the trend toward a decreased frequency of DRB1*15 in our study are consistent with the findings in a study from the UK that included 27 patients with juvenile-onset SSc with dermatomyositis overlap, although the frequency of DRB1*07 was not decreased in that study (7). Another HLA study included patients with localized pediatric-onset SSc, along with adults (9); although the results in the pediatric patients were not presented separately, none of the HLA associations with localized scleroderma were similar to findings in our study.

As previously shown in studies of adult patients with SSc, the current study of juvenile-onset SSc revealed an increased frequency of anti-topo I in patients with dcSSc and of ACA in patients with lcSSc. Interestingly, the prevalence of anti-topo I significantly decreased with increasing age at disease onset. The frequency of ACA was increased in children who were older at disease onset, but was low overall in juvenile-onset SSc compared to adult SSc, as has been described in other studies (4–6,10). Our ability to identify significant HLA associations with ACA and anti-topo I was limited; only half of the cohort members were positive for 1 of these auto-antibodies. However, DRB1*01 was significantly under-represented among patients with juvenile-onset SSc who were positive for anti-topo I, consistent with a similar observation in adult patients with SSc (18).

In summary, in the current study we identified HLA class II associations with juvenile-onset SSc, including one previously reported in adult male patients and dissimilar to findings in adult female patients. We also describe for the first time an association of juvenile-onset SSc with DRB1*10. Additionally, an age effect was observed for anti-topo I and ACA, as was an inverse correlation of DRB1*01 with anti-topo I. Overall, these results extend and confirm the importance of the HLA class II region in this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Odds ratios with 95% confidence intervals for associations of HLA class II allele groups with juvenile-onset systemic sclerosis versus healthy controls. Red and blue bars represent risk and protective associations, respectively, that have P values between 0.05 and 0.01 (except for DRB1*10, for which $P = 0.0002$ [$P = 0.003$ after correction for multiple comparisons]).

Figure 2.

Proportion of patients with juvenile-onset systemic sclerosis (jSSc) who were positive for HLA-DRB1*01 alleles, by age at onset and by presence of anticentromere antibodies (ACAs) and anti-topoisomerase I (anti-topo I). CTL = control.

Table 1.Clinical characteristics of Caucasian juvenile-onset SSc patients and Caucasian controls^{*}

	Juvenile-onset SSc				Controls		
	All (n = 76)	Seattle (n = 36)	Pittsburgh (n = 20)	Houston (n = 20)	All (n = 581)	Seattle (n = 176)	Houston (n = 405)
Sex, % female	87	83	95	85	48	46	49
Age, mean years [†]	10	8.9	11	11	39	16	49
Age, median (IQR) years	11.0 (6–13)	10.2 (5.9–11.9)	11.9 (9–14.1)	11.5 (8.5–13.6)	40 (24–56)	14 (8–23)	51 (37–61)
SSc subtype, %							
dcSSc	37	50	25	25	–	–	–
lcSSc	63	50	75	75	–	–	–
Autoantibodies (positive), %							
ANA	90.5	88.9	100	84.2	–	–	–
Anti-topo I	32.4	36.1	33.3	25.0	–	–	–
ACA	18.3	6.1	47.4	10.5	–	–	–

^{*} SSc = systemic sclerosis; IQR = interquartile range; dcSSc = diffuse cutaneous SSc; lcSSc = limited cutaneous SSc; ANA = antinuclear antibody; anti-topo I = anti-topoisomerase I; ACA = anticentromere antibody.

[†] Age at disease onset for juvenile-onset SSc patients; age at which blood was obtained for controls.