

## HLA–DRB1\*0407 and \*1304 Are Risk Factors for Scleroderma Renal Crisis

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**Objective.** To examine the predictive role of HLA genetic markers in scleroderma renal crisis (SRC), beyond the known clinical correlates, in a large population of patients with systemic sclerosis (SSc).

**Methods.** SSc patients from the Scleroderma Family Registry and DNA Repository, the Genetics versus Environment in Scleroderma Outcomes Study, and the rheumatology division registry at the University of Texas Health Science Center at Houston were included in the study. Relevant clinical data were obtained by chart review, and autoantibodies were detected utilizing commercially available kits. HLA class II genotyping was performed on extracted and purified genomic DNA.

**Results.** Overall, 1,519 SSc patients were included in the study, of whom 90 (6%) had developed SRC.

Among the 90 patients with SRC, the diffuse cutaneous disease subtype was found in 76%, antitopoisomerase antibodies (antitopo) in 9%, anticentromere antibodies (ACAs) in 2%, and anti–RNA polymerase III (anti–RNAP III) in 50% of patients. In multivariate analyses of clinical and demographic parameters, diffuse disease type and anti–RNAP III were strong risk factors for the presence of SRC, whereas ACAs and antitopo were protective. In the final multivariate analysis, which included HLA alleles, *HLA–DRB1\*0407* (odds ratio [OR] 3.21, 95% confidence interval [95% CI] 1.27–8.08;  $P = 0.013$ ) and *DRB1\*1304* (OR 4.51, 95% CI 1.30–15.65;  $P = 0.018$ ) were identified as independent risk factors for SRC. Only 3 clinical characteristics, diffuse disease type, anti–RNAP III, and ACAs, remained significantly associated with SRC in the final model.

**Conclusion.** The results of this study suggest that *DRB1\*0407* and *\*1304* are independent risk factors, beyond the known clinical correlates, for the development of SRC.

Systemic sclerosis (SSc) is an autoimmune disease of unclear etiology, characterized by fibrosis of the skin and internal organs, dysregulation in the immune system, and vasculopathy. SSc is a heterogeneous disease that can range from limited disease to extensive involvement with rapid progression, leading to disability and death. One of the life-threatening complications of SSc is scleroderma renal crisis (SRC). SRC is characterized by a rapid increase in blood pressure and renal failure. Other features of SRC can include microangiopathic hemolytic anemia and non–nephrotic-range proteinuria.

The prevalence of SRC in patients with SSc is ~5% (1). SRC is more likely to develop within 4 years of the first non-Raynaud's symptoms (2). It occurs frequently with diffuse disease and is correlated with rapid

Supported by NIH National Center for Research Resources Clinical and Translational Sciences grant UL1-RR-024148, National Institute of Arthritis and Musculoskeletal and Skin Diseases grant N01-AR0-2251, University Clinic Research Center grants M01-RR-00073 (University of Texas Medical Branch) and M01-RR-01346 (University of Texas–San Antonio), Department of Defense Congressionally Directed Medical Research Programs grant PR064251 (to Dr. Mayes), National Institute of Arthritis and Musculoskeletal and Skin Diseases Center of Research Translation in Scleroderma grant P50-AR-054144 (to Dr. Arnett), and grant KL2-RR-024149-04 (to Dr. Assassi).

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Dr. Mayes has received consulting fees, speaking fees, and/or honoraria from Actelion, Gilead, United Therapeutics, and Med-Immune (less than \$10,000 each).

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Submitted for publication July 10, 2010; accepted in revised form October 19, 2010.

progression of skin involvement (3). SRC is strongly associated with anti-RNA polymerase III autoantibodies (anti-RNAP III) (4), whereas it occurs rarely in patients with anticentromere antibodies (ACAs). Other possible risk factors for SRC are the use of prednisone (>15 mg), new cardiac events, and anemia (5). SRC-related mortality has been significantly decreased with the use of angiotensin-converting enzyme (ACE) inhibitors. In fact, pulmonary disease has become the leading cause of SSc-related mortality, replacing SRC, in recent years (6).

In the first large genome-wide association study of SSc, the major histocompatibility complex (MHC) region showed the strongest association with this disease. Furthermore, a recent study of a large multiethnic cohort of SSc patients showed that *HLA-DQA1\*0501* and *DQB1\*0301* were shared SSc-susceptibility alleles among all ethnic groups (7). In addition, our group has previously shown that *DQA1\*0501* and *DRB1\*0802* were significant predictors of mortality, beyond the known demographic and clinical predictors (8). However, no studies have evaluated the association of HLA alleles with SRC. Therefore, the purpose of this study was to examine the predictive role of HLA genetic markers, beyond the known clinical correlates, in the development of SRC in a large population of patients with SSc.

## PATIENTS AND METHODS

**Patient selection.** All study patients fulfilled the American College of Rheumatology preliminary criteria for SSc (9) or had 3 of the 5 clinical features of the CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasias). Patients were obtained from 3 sources: the Scleroderma Family Registry and DNA Repository (10), the Genetics versus Environment in Scleroderma Outcomes Study (GENISOS) cohort (11), and the rheumatology division registry at the University of Texas Health Science Center at Houston. Patients enrolled in more than one of the above-mentioned sources were identified, and duplicate entries were omitted. Renal crisis was defined as rapidly progressive renal failure and new-onset accelerated hypertension with or without microangiopathic hemolytic anemia. The comparison group was defined as SSc patients without SRC who were enrolled in the same cohorts as the cases. All study subjects provided written informed consent, and the study was approved by the University of Texas-Houston Committee for the Protection of Human Subjects.

**Autoantibody and genetic analyses.** Antinuclear antibodies (ANAs) were detected using indirect immunofluorescence on HEp-2 cells as the antigen substrate (Autoantibodies Inc.). A titer of  $\geq 1:80$  was considered to be positive. ACAs were determined by the pattern of immunofluorescence staining on HEp-2 cells. Antitopoisomerase antibodies (antitopo) were determined by passive immunodiffusion against calf

thymus extract with commercial kits (Inova Diagnostics). Anti-RNAP III autoantibodies were determined by enzyme-linked immunosorbent assay (MBL). HLA class II genotyping (*DRB1*, *DQA1*, *DPB1*) was performed on extracted and purified genomic DNA amplified through standard laboratory procedures, as previously described (12).

**Statistical analysis.** The outcome variable was the occurrence of SRC. The univariate comparisons were conducted by chi-square test for categorical variables and *t*-test for continuous independent variables. The multivariate model was constructed following a purposeful variable-selection method (13). First, we evaluated the demographic and clinical independent variables, without genetic risk factors, for their multivariable associations with the development of SRC, by logistic regression. All variables considered to be clinically important factors, along with those showing a significant association at *P* values less than 0.25, in the univariate analysis were included initially. In successive models, we eliminated, one-at-a-time, those covariates with the highest *P* value greater than 0.05, in order to reduce the initial saturated model. Reduced models were compared with each previous model to assess the potential for confounding, before eliminating a nonsignificant covariate. Those nonsignificant covariates whose exclusion changed the coefficients of the remaining covariates by >20% were retained as potentially important confounders. Covariates excluded from interim models were added back to the final model (one-at-a-time) to confirm their lack of both statistical significance and importance as a potential confounder.

For the analysis of the genetic data, univariate logistic regression models for each HLA allele were analyzed, categorized as heterozygous or homozygous per patient, to assess for dominant, recessive, or additive modes of risk inheritance. Ethnicities were included in all univariate and multivariate genetic analysis models. Subsequently, we conducted a separate, purposeful model-building analysis with inclusion of the genetic data, using the same procedure as described above.

Goodness-of-fit tests of both final multivariable models indicated good concurrence between the observed and the fitted values. The analysis was conducted with Stata Corporation statistical software, version 11.

## RESULTS

Overall, 1,519 patients with SSc were included in the study. The majority of patients were female and the mean  $\pm$  SD age at disease onset was  $44 \pm 14$  years, with a mean  $\pm$  SD disease duration of  $9 \pm 8$  years at enrollment. The patients consisted of the following ethnicities: 1,083 Caucasian (71%), 177 African American (12%), and 208 Hispanic (14%). A total of 686 patients (45%) had diffuse disease. ANA positivity was found in 92% of the patients, and anti-RNAP III was present in 18%. Ninety patients (5.9%) had SRC.

In the univariate analysis, anti-RNAP III (odds ratio [OR] 5.5, *P* < 0.001) and diffuse disease type (OR 3.9, *P* < 0.001) were strong risk factors for the presence

**Table 1.** Univariate analysis of demographic and clinical parameters and candidate HLA alleles in systemic sclerosis (SSc) patients with scleroderma renal crisis (SRC) in comparison with SSc patients without SRC\*

	SSc without SRC (n = 1,429)	SSc with SRC (n = 90)	OR	95% CI	P
Age at disease onset, mean $\pm$ SD years	44.2 $\pm$ 14.1	45.1 $\pm$ 11.1	1.00	0.99–1.02	0.57
Female	1,330 (93.1)	78 (86.7)	0.92	0.49–1.72	0.79
Disease duration, mean $\pm$ SD years	9.3 $\pm$ 8.1	8.3 $\pm$ 6.4	0.98	0.95–1.01	0.29
Ethnicity					
Caucasian	1,025 (71.7)	58 (64.4)	0.71	0.46–1.12	0.14
African American	161 (11.3)	16 (17.8)	1.76	0.99–3.13	0.06
Hispanic	193 (13.5)	15 (16.7)	1.37	0.76–2.47	0.29
Diffuse disease	618 (43.2)	68 (75.6)	3.9	2.39–6.41	<0.001
Autoantibodies†					
ACAs	304 (25.5)	2 (2.2)	0.06	0.02–0.26	<0.001
Antitopo	222 (18.6)	8 (8.8)	0.42	0.20–0.88	0.022
Anti-RNAP III	182 (15.3)	45 (50.0)	5.5	3.54–8.54	<0.001
RNP	80 (6.7)	6 (6.7)	1.08	0.50–2.76	0.71
<i>HLA-DRB1*0407</i> , no./total assessed‡	48/1,381	7/80	2.89	1.22–6.58	0.016
<i>HLA-DRB1*1304</i> , no./total assessed‡	18/1,381	4/80	4.04	1.27–12.92	0.018

\* Except where indicated otherwise, values are the number (%) of patients. OR = odd ratio; 95% CI = 95% confidence interval; ACAs = anticentromere antibodies; antitopo = antitopoisomerase antibodies; anti-RNAP III = anti-RNA polymerase III antibodies.

† Based on 1,191 patients for whom information was available.

‡ Adjusted for ethnicity.

of SRC, whereas ACAs (OR 0.06,  $P < 0.001$ ) and antitopo (OR 0.42,  $P = 0.022$ ) were protective. Sex, ethnicity, disease duration at enrollment, and RNP antibodies did not have a significant relationship to the presence of SRC (Table 1). Furthermore, the source of study subject recruitment was not associated with SRC ( $P = 0.819$ ).

All variables associated with SRC in the univariate analysis also were independent correlates of the outcome in the multivariable model. Specifically, anti-RNAP III (OR 2.72, 95% confidence interval [95% CI] 1.68–4.41;  $P < 0.001$ ) and diffuse disease (OR 2.10, 95% CI 1.25–3.55,  $P = 0.005$ ) were independent risk factors for SRC, whereas ACAs (OR 0.11, 95% CI 0.03–0.46,  $P = 0.003$ ) and antitopo (OR 0.42, 95% CI 0.19–0.91,  $P = 0.029$ ) were protective (Table 2).

In a separate multivariate analysis that included HLA alleles in addition to demographic and clinical variables, we identified *HLA-DRB1\*0407* (OR 3.21, 95% CI 1.27–8.08,  $P = 0.013$ ) and *\*1304* (OR 4.51, 95% CI 1.30–15.68,  $P = 0.018$ ) as independent risk factors for

**Table 2.** Multivariate logistic regression analysis of associations of scleroderma renal crisis with nongenetic risk factors\*

	OR	95% CI	P
ACAs	0.11	0.03–0.46	0.003
Antitopo	0.42	0.19–0.91	0.029
Anti-RNAP III	2.72	1.68–4.41	<0.001
Diffuse disease	2.10	1.25–3.55	0.005

\* See Table 1 for definitions.

SRC (Table 3). Only 3 clinical characteristics, anti-RNAP III, diffuse disease type, and ACAs, remained significantly associated with SRC in this final model. After taking into account genetic influence, antitopo no longer showed a statistically significant association (95% CI 0.23–1.14,  $P = 0.102$ ). Furthermore, the first-order interaction terms between the HLA types *DRB1\*0407* and *\*1304* and ethnicity in relation to development of SRC were not significant, indicating that these HLA types had a similar effect on the development of SRC across ethnic lines. As noted in Table 1, both HLA types, *DRB1\*0407* and *\*1304*, were also risk factors for SRC at the univariate level ( $P = 0.016$  and  $P = 0.018$ , respectively).

Homozygosity for these 2 alleles was not present in our patients. Therefore, we could not distinguish

**Table 3.** Multivariate logistic regression assessing associations with SRC with the addition of genetic risk factors in the model\*

	OR	95% CI	P
ACAs	0.15	0.03–0.63	0.010
Anti-RNAP III	3.24	1.96–5.38	<0.001
Diffuse disease	2.21	1.22–4.00	0.009
<i>HLA-DRB1*0407</i>	3.21	1.27–8.08	0.013
<i>HLA-DRB1*1304</i>	4.51	1.30–15.68	0.018
Ethnicity			
Caucasian	1.00		
African American	1.22	0.61–2.44	0.571
Asian	0.93	0.47–1.85	0.839
Hispanic	1.91	0.21–17.59	0.567

\* See Table 1 for definitions.

between a dominant and an additive inheritance mode of risk; however, our data did not suggest that a recessive inheritance mode of risk was present in these patients. *DRB1\*0407* was present in 2.9%, 2.7%, and 12.5% of Caucasian, African American, and Hispanic patients, respectively. The frequency of *DRB1\*1304* was 0.7%, 5.3%, and 1.8% in Caucasian, African American, and Hispanic patients, respectively.

## DISCUSSION

This study is the first to demonstrate an association of HLA alleles with SRC in a large cohort of patients with SSc. Both HLA alleles, *DRB1\*0407* and *\*1304*, were risk factors for SRC independent of clinical variables in the multivariate analysis. Confirming the findings from previous studies, diffuse cutaneous involvement and anti-RNAP III also were risk factors for SRC in our cohort (2–5).

Several studies have shown an association of prednisone usage prior to the development of SRC as a risk factor. Steen et al observed an association between the initiation of high-dose prednisone treatment (>15 mg daily) and SRC (5). This observation was also noted in a cohort of French patients receiving corticosteroid treatment at 1 or 3 months prior to the development of SRC (ORs of 17.4 and 24.1 for the likelihood of developing SRC, respectively) (14). Data on prednisone use prior to the development of SRC were not available in our study.

SSc-related autoantibodies are associated with particular SSc clinical characteristics. These autoantibodies are usually mutually exclusive. Anti-RNAP III are highly specific for SSc and occur more frequently in patients with the diffuse disease subtype, and also are associated with SRC (4). Our study confirms the association of anti-RNAP III with SRC. In our study, the presence of antitopo was negatively associated with SRC. ACAs are associated with limited cutaneous disease and isolated pulmonary hypertension. In fact, SRC rarely occurs in SSc patients with ACA antibodies. Consistent with the findings in previous studies, the results of the present study showed that ACAs were protective, with an OR of 0.05, indicating a reduced likelihood of developing SRC (1).

With the use of ACE inhibitors, mortality and morbidity related to the presence of SRC have significantly decreased. However, prophylactic use of ACE inhibitors has not been shown to prevent SRC. In several studies, patients were observed to develop SRC despite having received treatment with ACE inhibitors (14,15). Therefore, we do not believe that many cases of SRC

were masked in our study because of concomitant use of ACE inhibitors.

Previous studies have also evaluated the influence of MHC genes on mortality, disease susceptibility, and autoantibody expression among the different ethnic groups. Assassi et al investigated 250 patients with SSc for clinical and genetic characteristics predictive of mortality (8). Seven clinical characteristics were associated with mortality: age  $\geq 65$  years, forced vital capacity <50% of the predicted value, clinically significant arrhythmia on electrocardiogram, absence of ACAs, low body mass index, hypertension, and pulmonary fibrosis on chest radiograph. In the multivariate analysis with inclusion of genetic factors, the HLA alleles *DRB1\*0802* and *DQA1\*0501* were found to be predictors of mortality independent of the above clinical characteristics. These findings further support a role for genetic biomarkers in the prediction of disease outcome in SSc.

Arnett et al evaluated the association between MHC alleles and SSc susceptibility and their influences on expression of autoantibodies in a large multiethnic SSc cohort (Caucasian, African American, and Hispanic) (7). The study found a significant association of both shared and unique alleles between different ethnicities and disease susceptibility. *HLA-DQA1\*0501* and *DQB1\*0301* were shared SSc susceptibility alleles among all ethnic groups. In addition to conferring disease susceptibility, MHC genes were associated with autoantibody expression. *DPB1\*1301* was significantly associated with antitopo among all ethnicities. Anti-RNAP III was associated with *DRB1\*0404*, *DQB1\*0301*, and *DRB1\*1104* alleles in Caucasians, *DRB1\*0804*, *DQA1\*0501*, and *DQB1\*0301* in African Americans, and *DRB1\*11*, *DQA1\*0501*, and *DQB1\*0301* in Hispanics (7). This study demonstrated that HLA genotype influenced disease susceptibility and was associated with autoantibody expression that was both shared and unique among the different ethnicities.

Our study is the first study to demonstrate an association of *DRB1\*0407* and *\*1304* with SRC. Patients with these HLA genotypes had a likelihood of developing SRC similar to that in association with the presence of anti-RNAP III. As mentioned above, certain HLA alleles increased the susceptibility to SSc and were associated with autoantibody expression. We considered the possibility that *DRB1\*0407* and *\*1304* exerted their influences through anti-RNAP III expression. However, in multivariate analysis, the effects of these alleles were not diminished by inclusion of anti-RNAP III. Similarly, Arnett et al did not show any association of *DRB1\*0407* and *\*1304* with the expression of anti-RNAP III (7). Therefore, the predictive significance of *DRB1\*0407*

and \*1304 was independent of autoantibody status. *DRB1\*0407* is an allele predominant in American Indians, and *DRB1\*1304* is African in origin, although these HLA types were also present, to a lesser extent, in other ethnicities in our study population, raising the possibility of occult ethnic admixture. It also needs to be explored whether occult ethnic admixture has a role in susceptibility to SRC.

It is important to emphasize that any concern regarding the effects of multiple comparisons is related to the increased risk of erroneous associations being reported as the number of separate univariate analyses (i.e., separate hypothesis tests) increases. In contrast, our goal with the use of multivariable modeling was to identify the single best set of independent risk factors, by utilizing a multivariable model in which candidate variables were examined in simultaneous combination. Therefore, the influence of each variable on the outcome was adjusted for the effect of all other covariates in the model. In such a multivariate analysis, the adjustment of *P* values for the number of comparisons (i.e., multiple hypothesis tests) is not relevant, because only a single test of the overall model is utilized to evaluate the relationship of the independent variables, as a group, with the investigated outcome.

One limitation of the current study was that 2 of the 3 sources investigated were cross-sectional databases, raising the possibility of missing SRC cases if the event developed after study enrollment. However, the prevalence of SRC was not significantly different between the cross-sectional samples and the longitudinal cohort.

Results of the present study suggest that HLA genetic data, specifically *DRB1\*0407* and \*1304, can be used to identify patients at risk for the development of SRC. However, these results need to be confirmed in an independent cohort. Furthermore, these genetic markers might be useful as predictive biomarkers only in populations of patients in whom the frequency of these 2 alleles is relatively high.

SRC can cause significant morbidity and mortality. Identification of patients at risk for SRC enables clinicians to implement individualized approaches for monitoring the development of this condition. Our results indicate that *HLA-DRB1\*0407* and \*1304 are independent correlates of SRC, beyond the known clinical risk factors. In populations with a high frequency of *HLA-DRB1\*0407* and \*1304, they could potentially be used as predictive markers for SRC.

## 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. Assassi 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.

**Study conception and design.** Nguyen, Mayes, Arnett, del Junco, Reveille, Draeger, Anand, Assassi.

**Acquisition of data.** Nguyen, Mayes, Arnett, Reveille, Gonzalez, Draeger, Perry, Hendiani, Assassi.

**Analysis and interpretation of data.** Nguyen, Mayes, Arnett, del Junco, Reveille, Draeger, Hendiani, Anand, Assassi.

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