Published in final edited form as:
Arthritis Rheum. 2011 February ; 63(2): 530–534. doi:10.1002/art.30111.

HLA-DRB1*0407 and *1304 are Risk Factors for Scleroderma Renal Crisis

Binh Nguyen, MD[1], **Maureen D. Mayes, MD, MPH**[1], **Frank C. Arnett, MD**[1], **Deborah del Junco, PhD**[2], **John D. Reveille, MD**[1], **Emilio B. Gonzalez, MD**[3], **Hilda T. Draeger, MD**[4], **Marilyn Perry, BS**[1], **Amir Hendiani, MD**[1], **Kiran K. Anand, MD**[1], and **Shervin Assassi, MD, MS**[1]

[1]Division of Rheumatology and Clinical Immunogenetics, University of Texas-Houston, Texas

[2]Center for Clinical and Translational Studies, University of Texas-Houston, Texas

[3]Division of Rheumatology, University of Texas Medical Branch at Galveston, Texas

[4]Division of Rheumatology, University of Texas-San Antonio, Texas

Abstract

OBJECTIVE—To examine the predictive role of HLA genetic markers for 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, Genetics versus Environment in Scleroderma Outcomes Study (GENISOS) and rheumatology divisional 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, 1519 SSc patients were included in this study, from which 90 patients (6%) had developed SRC. Among the 90 patients with SRC, diffuse cutaneous subtypes were found in 76%, anti-toposiomerase (ATA) in 9%, anti-centromere antibodies(ACA) in 2%, and anti-RNA polymerase III (ARA) in 50% of patients. In the multivariate analysis of clinical and demographic parameters, diffuse disease type and ARA were strong risk factors for presence of SRC, whereas ACA and ATA were protective. In the final multivariate analysis after inclusion of HLA alleles, we identified *HLA-DRB1*0407* (OR=3.21, 95% CI 1.27–8.08; P=0.013) and *DRB1*1304* (OR=4.51, 95% CI 1.30–15.65; P=0.018) as independent risk factors for SRC. Only 3 clinical characteristics, diffuse disease type, ARA, and ACA remained significant in the final model.

CONCLUSION—This study suggests that *DRB1*0407* and **1304* are independent risk factors for development of SRC beyond the known clinical correlates.

Systemic sclerosis (SSc) is an autoimmune disease of unclear etiology characterized by fibrosis in 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 rapid increase in blood pressure and renal failure. Other features of SRC can include microangiopathic hemolytic anemia and non-nephrotic range proteinuria.

Correspondence to: Shervin Assassi, MD, MS, University of Texas Health science Center at Houston, 6431 Fannin, MSB 5.270, Houston, TX 77030, USA, Phone: (713) 500-6900, Fax: (713) 500-0580, shervin.assassi@uth.tmc.edu.

The prevalence of SRC in patients with SSc is about 5% (1), SRC is more likely to present within four years of the first non-Raynaud's symptoms (2). It occurs frequently with diffuse disease and correlates with rapid skin progression (3). SRC is strongly associated with anti-RNA polymerase III (ARA) autoantibodies (4) whereas it occurs rarely in patients with anti-centromere antibodies (ACA). Other possible risk factors for SRC are prednisone usage (>15 mg), new cardiac events, and anemia (5). SRC related mortality has significantly improved 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 histocompatibililty 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 reported 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.

The purpose of this study was to examine the predictive role of HLA genetic markers for SRC beyond the known clinical correlates 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 dysfunction, Sclerodactyly or Telangiectasia). Patients were obtained from three sources: the Scleroderma Family Registry and DNA Repository (10) the Genetics versus Environment in Scleroderma Outcomes Study (GENISOS) cohort (11) and the rheumatology divisional 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 UTH Committee for the Protection of Human Subjects.

Autoantibody and genetic analysis

ANA was detected using indirect immunofluorescence on HEp-2 cells as the antigen substrate (Autoantibodies Inc, Davis, CA, USA). A titer of 1:80 or more was considered to be positive. ACA was determined by the pattern of immunofluorescence staining on HEp-2 cells. Anti-topoisomerase (ATA) was determined by passive immunodiffusion against calf thymus extract with commercial kits (Inova Diagnostics, San Diego, CA, USA). Anti-RNA polymerase III (ARA) was determined by enzyme-linked immunoassay (MBL Co. Ltd, Nagoya, Japan).

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 and t-test for continuous independent variables. 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 renal crisis by logistic regression. All variables considered to be clinically important variables along with those reaching P-values < 0.25 in the univariate analysis were initially included. Successive models eliminated one-at-a-time 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. 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 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 after inclusion of the genetic data according to the above described procedure.

Goodness of fit tests of both final multivariable models indicated good fit between the observed and fitted values. The analysis was conducted with the Stata 11 (StataCorp LP, College Station, TX, USA) statistical software.

Results

Overall, 1519 patients were included in the study. The majority of patients were female and the mean age at disease onset (±SD) was 44 ± 14 years with the average disease duration (±SD) of 9 ± 8 years at enrollment. The patients consisted of the following ethnicities: 1083 Caucasians (71%), 177 African-Americans (12%), and 208 Hispanics (14%). A total of 686 patients (45%) had diffuse disease. ANA positivity was found in 92% of the patients and ARA was present in 18% of the cohorts. Ninety patients (5.9%) had SRC.

In the univariate analysis, ARA (p<0.001, OR=5.5) and diffuse disease type (p<0.001, OR=3.9) were strong risk factors for presence of SRC, whereas ACA (p<0.001, OR= 0.06) and ATA (p=0.022, OR=0.42) were protective. Gender, ethnicity, disease duration at enrollment and RNP antibodies did not have a significant relationship to SRC. 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, ARA [(P<0.001; OR=2.72 (1.68–4.41)] and diffuse disease [(P=0.005; OR=2.10 (1.25–3.55)] were independent risk factors of SRC, whereas ACA [(P=0.003; OR=0.11, (0.03–0.46)] and ATA [(P=0.029; OR=0.42, (0.19–0.91)] were protective.

In a separate multivariate analysis with demographic, clinical and HLA alleles (Table 3), 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 SRC. Only 3 clinical characteristics, ARA, diffuse disease type and ACA remained in this final model. After taking into account for genetic influence, ATA was no longer statistically significant (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 for development of SRC were not significant, indicating that these HLA types had a similar effect across the ethnic lines on the development of SRC.

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 two alleles was not present in our patients. Therefore, we could not distinguish between dominant and additive inheritance mode of risk but our data did not suggest recessive inheritance mode. 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 Caucasians, African Americans and Hispanics, respectively.

Discussion

This study demonstrates for the first time an association of HLA alleles with SRC in a large systemic sclerosis cohort. Both HLA alleles, *DRB1*0407* and **1304*, were risk factors for SRC independent of clinical variables in the multivariate analysis. Confirming previous studies, diffuse cutaneous involvement and ARA also were risk factors for SRC in our cohort (2,3,4,5).

Several studies have shown an association of prednisone usage prior to SRC as a risk factor. Steen *et al.* observed an association between initiation of high dose prednisone treatment (> 15 mg daily) and SRC (5). This observation was also noted in a French cohort demonstrating patients receiving corticosteroid 1- or 3-month prior to event had OR of 17.4 and 24.1 for developing SRC, respectively(14). Data about prednisone use prior to 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. ARA is highly specific for SSc and occurs more frequently in patients with diffuse disease subtype and is associated with SRC (4). Our study confirms the association of ARA with SRC. In our study, ATA was negatively associated with SRC. ACA is associated with limit cutaneous disease and isolated pulmonary hypertension. In fact, SRC rarely occurs in SSc patients with ACA antibodies. In agreement with previous studies, the current study indicated that ACA was “protective” with an OR of 0.05 against developing SRC (1).

With the use of ACE inhibitors, mortality and morbidity of SRC has significantly decreased. However, prophylactic use of ACE inhibitors has not been shown to prevent SRC. Several studies observed patients developing SRC despite being on 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 had evaluated MHC genes influencing mortality, disease susceptibility and autoantibody expression among the different ethnic groups. Assassi *et al.* investigated 250 SSc patients for clinical and genetic characteristics predictive of mortality (7). Seven clinical characteristics were associated with mortality: age ≥ 65 years, FVC < 50% of the predicted value, clinically significant arrhythmia on EKG, absence of ACAs, low BMI, hypertension, and pulmonary fibrosis on chest radiograph. In multivariate analysis with genetic influences, HLA alleles, *DRB1*0802* and *DQA1*0501*, were predictors of mortality independent of the above clinical characteristics. These findings further supported a role for genetic biomarkers for predicting disease outcome in SSc. Arnett *et al.* evaluated association of MHC alleles and SSc susceptibility and their influences on expression of autoantibodies in a large SSc multiethnic cohort (Caucasian, African-American and Hispanics) (6). The study found

significant association of both shared and unique alleles between different ethnicities and diseases 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 expressions. *DPB1*1301* was significantly associated with ATA among all ethnicities. ARA was associated with *DRB1*0404*, *DQB1*0301*, and *DRB1*1104* allele in Caucasians and *DRB1*0804*, *DQA1*0501*, and *DQB1*0301* in African-American and *DRB1*11*, *DQA1*0501*, and *DQB1*0301* in Hispanics. 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 association of *DRB1*0407* and **1304* with SRC. Patients with these HLA genotypes had odd ratios of developing SRC similar to the presence of ARA. As mentioned above, certain HLA alleles increased susceptibility to SSc and were associated with autoantibody expression. We considered the possibility that *DRB1*0407* and **1304* exerted their influences through ARA expression. However, in multivariate analysis, the effects of these alleles were not diminished by inclusion of ARA. In addition, Arnett *et al*. did not show any association of *DRB1*0407* and **1304* with expression of ARA (6). Therefore, the predictive significance of *DRB1*0407* and **1304* was independent of autoantibody status. *DRB1*0407* is mainly an American Indian allele 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 the concern for multiple comparisons relates to the increased risk of reporting erroneous associations as the number of separate univariate analyses (i.e., separate hypothesis tests) increased. In contrast, our goal was to identify the single best set of independent risk factors using multivariable modeling, in which candidate variables were examined in simultaneous combination. Therefore, each variable's influence on the outcome was adjusted for the effect of all the 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 report was that two of three investigated sources 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.

The current study suggests that HLA genetic data, specifically *DRB1*0407* and **1304*, can be used to identify patients at risk for development of SRC. However, these results need to be confirmed in an independent cohort. Furthermore, they might be only useful as predictive biomarkers in populations where the frequency of these two alleles is relatively high.

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

References

1. Penn H, Howie AJ, Kingdon EJ, Bunn CC, Stratton RJ, Black CM, et al. Scleroderma renal crisis: patient characteristics and long-term outcomes. QJM. 2007; 100:485–494. [PubMed: 17601770]
2. Bunn CC, Denton CP, Shi-wen X, Knight C, Black CM. Anti-RNA polymerases and other autoantibody specificities in systemic sclerosis. Br J Rheumatol. 1998; 37:15–20. [PubMed: 9487245]
3. DeMarco PJ, Weisman MH, Seibold JR, Furst DE, Wong WK, Hurwitz EL, et al. Predictors and outcomes of scleroderma renal crisis: the high-dose versus low-dose D-penicillamine in early diffuse systemic sclerosis trial. Arthritis Rheum. 2002; 46:2983–2989. [PubMed: 12428241]
4. Okano Y, Steen VD, Medsger TA Jr. Autoantibody reactive with RNA polymerase III in systemic sclerosis. Ann Intern Med. 1993; 119:1005–1013. [PubMed: 8214977]
5. Steen VD, Medsger TA, Osial TA, Ziegler GL, Shapiro AP, Rodnan GP. Factors predicting development of renal involvement in progressive systemic sclerosis. Am J Med. 1984; 76:779–786. [PubMed: 6372452]
6. Steen VD, Medsger TA. Changes in causes of death in systemic sclerosis, 1972–2002. Ann Rheum Dis. 2007; 66:940–944. [PubMed: 17329309]
7. Arnett FC, Gourh P, Shete S, Ahn CW, Honey RE, Agarwal SK, et al. Major histocompatibility complex (MHC) class II alleles, haplotypes and epitopes which confer susceptibility or protection in systemic sclerosis: analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls. Ann Rheum Dis. 2010; 69:822–827. [PubMed: 19596691]
8. Assassi S, Del Junco D, Sutter K, McNearney TA, Reveille JD, Karnavas A, et al. Clinical and genetic factors predictive of mortality in early systemic sclerosis. Arthritis Rheum. 2009; 61:1403–1411. [PubMed: 19790132]
9. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum. 1980; 23:581–590. [PubMed: 7378088]
10. Mayes MD. The establishment and utility of a population-based registry to understand the epidemiology of systemic sclerosis. Curr Rheumatol Rep. 2000; 2:512–516. [PubMed: 11123106]
11. Reveille JD, Fischbach M, McNearney T, Friedman AW, Aguilar MB, Lisse J, et al. Systemic sclerosis in 3 US ethnic groups: a comparison of clinical, sociodemographic, serologic, and immunogenetic determinants. Semin Arthritis Rheum. 2001; 30:332–346. [PubMed: 11303306]
12. Olsen ML, Arnett FC, Reveille JD. Contrasting molecular patterns of MHC class II alleles associated with the anti-Sm and anti-RNP precipitin autoantibodies in systemic lupus erythematosus. Arthritis Rheum. 1993; 36:94–104. [PubMed: 7678744]
13. Hosmer, DW.; Lemeshow, S. Applied survival analysis: regression modeling of time to event data. New York: John Wiley & Sons; 1999. p. 159-180.
14. Teixeira L, Mouthon L, Mahr A, Berezné A, Agard C, Mehrenberger M, et al. Group Français de Recherche sur le Sclérodermie (GFRS). Mortality and risk factors of scleroderma renal crisis: a French retrospective study of 50 patients. Ann Rheum Dis. 2008; 67:110–116. [PubMed: 17557890]
15. Steen VD, Medsger TA. Case-control study of corticosteroids and other drugs that either precipitate or protect from the development of scleroderma renal crisis. Arthritis Rheum. 1998; 41:1613–1619. [PubMed: 9751093]

Table 1

Univariate comparisons of demographic and clinical parameters and candidate HLA alleles between SSc patients with and without SRC

	SSc without SRC (N=1429)	SSc with SRC (N=90)	OR	95% CI	P value
Age of onset	44.2 ± 14.1	45.1 ± 11.1	1.00	0.99–1.02	0.57
Female	93.1% (1330)	86.7% (78)	0.92	0.49–1.72	0.79
Disease duration	9.3 ± 8.1	8.3 ± 6.4	0.98	0.95–1.01	0.29
Ethnicity					
Caucasian	71.7% (1025)	64.4% (58)	0.71	0.46–1.12	0.14
African-American	11.3% (161)	17.8% (16)	1.76	0.99–3.13	0.06
Hispanics	13.5% (193)	16.7% (15)	1.37	0.76–2.47	0.29
Diffuse disease	43.2% (618)	75.6% (68)	3.9	2.39–6.41	<0.001
ACA†	25.5% (304)	2.2% (2)	0.06	0.02–0.26	<0.001
ATA†	18.6% (222)	8.8% (8)	0.42	0.20–0.88	0.022
ARA†	15.3% (182)	50.0% (45)	5.5	3.54–8.54	<0.001
RNP†	6.7% (80)	6.7% (6)	1.08	0.50–2.76	0.71
*HLA-DRB1*0407*‡	48/1381	7/80	2.89	1.22–6.58	0.016
*HLA-DRB1*1304*‡	18/1381	4/80	4.04	1.27–12.92	0.018

SRC= Scleroderma Renal Crisis; OR = odd ratio; 95% CI = 95% confidence interval; ACA = anti-centromere; ATA = anti-topoisomerase antibody; ARA = anti-RNA polymerase III antibody; RNP = anti-Ribonucleoprotein antibody.

†Based on 1191 patients whom we have information available.

‡Adjusted for ethnicity.

Table 2

Multivariate logistic regression with non-genetic risk factors

	OR	95% CI	P value
ACA	0.11	0.03–0.46	0.003
ATA	0.42	0.19–0.91	0.029
ARA	2.72	1.68–4.41	<0.001
Diffuse	2.10	1.25–3.55	0.005

OR = odd ratio; 95% CI = 95% confidence interval; ACA = anti-centromere; ATA = anti-topoisomerase antibody; ARA = anti-RNA polymerase III antibody.

Table 3

Multivariate logistic regression including genetic risk factors

	OR	95% CI	P value
ACA	0.15	0.03–0.63	0.010
ARA	3.24	1.96–5.38	<0.001
Diffuse	2.21	1.22–4.00	0.009
*HLA-DRB1*0407*	3.21	1.27–8.08	0.013
*HLA-DRB1*1304*	4.51	1.30–15.68	0.018
Ethnicity			
White	1		
African American	1.22	0.61–2.44	0.571
Asian	0.93	0.47–1.85	0.839
Hispanics	1.91	0..21–17.59	0.567

OR = odd ratio; 95% CI = 95% confidence interval; ACA = anti-centromere; ARA = anti-RNA polymerase III antibody.