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HLA Polymorphisms in African Americans With Idiopathic Inflammatory Myopathy

Allelic Profiles Distinguish Patients With Different Clinical Phenotypes and Myositis Autoantibodies

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Objective. **To investigate possible associations of HLA polymorphisms with idiopathic inflammatory myopathy (IIM) in African Americans, and to compare this with HLA associations in European American IIM patients with IIM.**

Methods. **Molecular genetic analyses of HLA–A, B, Cw, DRB1, and DQA1 polymorphisms were performed in a large population of African American patients with IIM (n = 262) in whom the major clinical and autoantibody subgroups were represented. These data were compared with similar information previously obtained from European American patients with IIM (n = 571).**

Results. **In contrast to European American patients with IIM, African American patients with IIM, in particular those with polymyositis, had no strong disease associations with HLA alleles of the 8.1 ancestral haplotype; however, African Americans with dermatomyositis or with anti–Jo-1 autoantibodies shared the risk factor HLA–DRB1*0301 with European Americans. We detected novel HLA risk factors in African American patients with myositis overlap (DRB1*08) and in African American patients producing anti–signal recognition particle (DQA1*0102) and anti–Mi-2 autoantibodies (DRB1*0302). DRB1*0302 and the European American–, anti–Mi-2–associated risk factor DRB1*0701 were found to share a 4–amino-acid sequence motif, which was predicted by comparative homology analyses to have identical 3-dimensional orientations within the peptide-binding groove.**

Conclusion. **These data demonstrate that North American IIM patients from different ethnic groups have both shared and distinct immunogenetic susceptibility factors, depending on the clinical phenotype. These findings, obtained from the largest cohort of North American minority patients with IIM studied to**

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date, add additional support to the hypothesis that the myositis syndromes comprise multiple, distinct disease entities, perhaps arising from divergent pathogenic mechanisms and/or different gene–environment interactions.

Idiopathic inflammatory myopathies (IIMs) comprise a heterogeneous group of autoimmune diseases with the primary features of muscle weakness and inflammation of unknown cause (1). These systemic autoimmune syndromes have clinical manifestations of symmetric, proximal muscle weakness, elevated serum levels of muscle enzymes, myopathic changes on electromyography, and features of inflammation on muscle biopsy. The 2 major clinicopathologic groups of IIM, dermatomyositis (DM) and polymyositis (PM), are distinguished clinically by the presence of skin photosensitivity, as well as pathognomonic rashes in DM. The IIM syndromes can be divided further into multiple serologic groups based on the presence of myositis-specific autoantibodies and myositis-associated autoantibodies, which are often associated with different epidemiologic, clinical, prognostic, and immunogenetic features (1,2).

Autoimmune diseases appear to develop after chronic immune activation in genetically susceptible individuals, following specific environmental exposures. This concept in relation to myositis is supported by familial clustering, immunogenetic associations with IIM, and temporal associations of disease onset with drugs and other environmental agents in certain individuals (1,3,4). Certain polymorphic genes of the human major histocompatibility complex (MHC) have been associated with myositis. These include HLA class I genes (HLA–A, B, and Cw) and HLA class II genes (HLA–DR, DQ, and DP), which encode antigen-presenting molecules that play important regulatory roles in immune activation. HLA genes are among the strongest and most consistently identified genetic factors associated with the development of human autoimmune diseases, including IIM (2,5–9). The relationship between MHC genetic variability and autoimmune disease may be explained, in part, by influences of the MHC molecules on T cell receptor development, peripheral tolerance, and immune responses to environmental agents (8,10,11).

We recently described distinct patterns of the HLA–A, B, Cw, DRB1, and DQA1 alleles and peptide-binding motifs associated with different clinicopathologic and serologic (i.e., myositis autoantibody) phenotypes among IIM patients of European American ancestry (9,12). Collectively, these and other findings suggest that multiple, distinct pathogenic mechanisms may contribute to the heterogeneity of IIM, and that recognition of genotypes may permit better stratification of IIM into more homogeneous groups of patients for study. Few studies have examined HLA allelic associations among African American patients with IIM (13–16). Therefore, we examined the allelic variability of HLA–A, B, Cw, DRB1, and DQA1 determinants in a large population of African American patients with IIM to assess genetic susceptibility in different clinicopathologic and autoantibody groups, and to compare these findings with the risk of and protective factors for IIM in European Americans.

PATIENTS AND METHODS

Study subjects. African American patients with adult-onset (age at onset >17 years; n = 207) and childhood-onset (n = 55) myositis and healthy, unrelated, ethnically matched control subjects (n = 311) were identified for this study from among individuals who were referred to protocols involving the pathogenesis and treatment of myositis at the NIH Warren Grant Magnuson Clinical Center and the US Food and Drug Administration between 1983 and 2002 (107 IIM cases and 198 controls). Additional case and control data from this period were provided from the University of Texas Houston Health Science Center (84 IIM cases and 92 controls), Mayo Clinic Rochester (4 IIM cases), and the University of Pittsburgh Medical Center (12 IIM cases and 21 controls) (17,18). The data from 55 patients with juvenile-onset myositis were provided by contributors in the Childhood Myositis Heterogeneity Study Group (13 patients with PM, 32 with DM, and 10 with myositis overlap). All subjects were enrolled in investigational review board–approved clinical protocols.

Patients were included in the case group if they met the criteria for probable or definite PM or DM (19) or inclusion body myositis (IBM) (2). All patients with inherited, metabolic, or infectious myopathies and other causes of muscle disease were excluded. Patients were categorized in the myositis overlap group if they met the criteria for probable or definite PM or DM or IBM and also the criteria for another defined connective tissue disease (CTD). Cancer-associated myositis (CAM) was defined when cancer was diagnosed within 2 years of the diagnosis of myositis. Altogether, 201 African American women and 61 African American men were evaluated in the case group; this female-to-male ratio reflects the predominance of women generally reported in other IIM cohorts (~3:1). A total of 167 African American patients with PM, 89 with DM, and 5 with IBM were studied, and of these, 63 had myositis/CTD overlap and 7 had CAM; the clinical subgroup of an additional patient having myositis overlap with another CTD was undefined. Data from some of these African American subjects have been published previously (2,15). African American patients were compared with a cohort of 571 European American patients with IIM who were recruited at the same institutions and who were phenotypically and genetically defined in the same way, as previously described (4,9).

Laboratory procedures. Low-to-high–resolution genotyping of all presently identified class I MHC (HLA–A, B, and

Cw) and class II MHC (HLA–DRB1 and DQA1) alleles was performed as described previously (9). HLA–DRB1 restrictive supertype patterns (RSPs) were screened by comparing case and control groups for the combined frequency of all alleles possessing the putative peptide-binding motifs (20). In addition, we described an anti–Mi-2–associated second hypervariable region (HVR2) motif in DRB1 that was defined by alleles DRB1*0302, *0701, *1402, and *1403. Myositis-specific (antisynthetase, anti–signal recognition particle [anti-SRP], and anti–Mi-2) and myositis-associated (anti-Ku, anti-La, anti-Ro, anti-RNP, and anti-PM/Scl) autoantibodies were identified in serum samples using previously validated methods of protein and RNA immunoprecipitation and double immunodiffusion (21,22).

Statistical analysis. Analyses were performed using SAS software for Windows, version 8.02 (SAS Institute, Cary, NC) as described previously (9). Multiple comparison corrections were performed using the sequential Holm procedure (23). The relative importance (RI) that individual HLA alleles confer upon genetic predisposition to disease was estimated using a Random Forests statistical learning algorithm (9) developed by Breiman and Cutler (24) (for more details, see the Web site http://stat-www.berkeley.edu/users/breiman/RandomForests/). Briefly, the Random Forests algorithm is a prediction and classification tool that generates rank estimates of variable importance and approximates case proximities within clusters. All alleles with complete HLA–DRB1 and DQA1 high-resolution typing data from the serologic subgroups of IIM patients with anti–Jo-1 or anti–Mi-2 and the control group were classified using Random Forests models with 1,000 independent classification trees. All allelic variables in the test population were ranked by their RI in terms of their ability to discriminate case and control test subjects. RI values (expressed as a percentage) were normalized to the highest-ranking factor (Gini score) in a given analysis; Gini scores were calculated using the Gini impurity criterion for individual variables over all classification trees in the Random Forests model. The results presented were obtained from analyses in which adequate sample sizes and satisfactorily low error rates permitted a reliable ranking of RI scores. We also performed traditional logistic regression analyses as an independent means of corroborating our Random Forests modeling.

Molecular modeling. Modeling of DRB1 protein structural homology was performed using DEEPVIEW Swiss-Pdb Viewer software, version 3.7 (see http://www.expasy.ch/spdbv/). DRB1*0302 and *0701 primary amino acid sequences were independently submitted to the automated comparative protein modeling server SWISS-MODEL (see http://swissmodel.expasy.org/) (25,26). Query sequences were threaded by alignment on composite templates (PDB identifiers for DRB1*0302: 1A6A, 1SEB, 1R5I, and 1PYW; for DRB1*0701: 1HXY, 1AQD, 1DLH, 1SJE, and 1SEB) using first approach mode (see http://www.rcsb.org/pdb/). Provisional structural alignments were subsequently refined within DEEPVIEW and resubmitted to SWISS-MODEL in optimize mode. The resulting DRB1 models were structurally aligned and optimized within SWISS-MODEL to produce a final composite 3-dimensional (3-D) structure of the DRB1*0301, *0302, and *0701 molecules (27).

RESULTS

Overview of the study population. The frequencies of IIM-specific clinicopathologic and serologic phenotypes seen in African American IIM patients were generally similar to those reported in European American IIM populations (Table 1). PM was the most prevalent clinicopathologic group detected among the African American patients with IIM (~62%, including patients with PM/CTD overlap), while DM and DM/CTD overlap comprised ~33% of the case group. As expected, IBM was significantly less frequent in the African American patients with IIM (1.9%) than in the European American patients with IIM (7.8%; $P = 0.0001$) (12).

Myositis-specific and myositis-associated autoantibodies were detected among ~47% and ~39% of the 242 African American patients with IIM surveyed, respectively (Table 1). Antisynthetase autoantibodies were the most frequently detected myositis-specific antibodies (26%), and anti–Jo-1 comprised 16% of the antisynthetase group. Of interest, higher frequencies of anti–PL-12 autoantibodies were detected among the African American IIM patients (5.0%) compared with the European American IIM patients (1.8%) ($P = 0.017$) (12). Anti-OJ autoantibodies had the lowest frequency among all of the myositis-specific antibodies reported in African Americans (0.8%). The majority (85%) of anti–Mi-2 autoantibody–positive African American patients had DM, and 94% of anti-SRP autoantibody–positive African American patients had PM. Higher frequencies of anti-SRP autoantibodies were detected among African American IIM patients (13.2%) compared with European American IIM patients (3.6%) ($P < 0.0001$), as observed in prior studies (12).

Myositis-associated autoantibodies were detected among 67% of African American patients with myositis/CTD overlap, consistent with the broader spectrum of CTDs represented in the myositis overlap group. Higher frequencies of both anti-Ro and anti-RNP autoantibodies were also observed among African American IIM patients (18%) compared with European American IIM patients (10% [$P = 0.002$] and 5% [$P < 0.0001$], respectively). In contrast, lower frequencies of anti-PM/Scl autoantibodies were detected among African American patients with IIM (2.5%) compared with their European American counterparts (11%) ($P < 0.0001$).

Analyses examining the coincident detection of individual myositis-specific and myositis-associated autoantibodies in African American patients with IIM revealed that no patients had more than 1 myositis-specific

Table 1. Autoantibody serogroups of African American patients with idiopathic inflammatory myopathies (IIMs)*

		IIM subgroup, no. (%)†		Clinical subgroup, no. (%)‡	
	All IIM, no. (%)	PM (n = 115)	DM (n = 73)	Myositis/CTD overlap (n = 63)	CAM (n = 7)
MSAs					
All MSAs	113 (46.7)	67 (60.4)	29 (46.8)	15 (25.9)	2 (28.6)
Antisynthetases	64 (26.4)	31 (27.9)	16 (28.8)	15 (25.9)	2 (28.6)
Anti–Jo-1	39 (16.1)	20 (18.0)	8 (12.9)	9 (15.5)	2 (28.6)
Anti–PL-7	6 (2.5)	2 (1.8)	1 (1.6)	3 (5.2)	0
Anti–PL-12	12 (5.0)	6 (5.4)	5 (8.1)	1 (1.7)	0
Anti-OJ	2 (0.8)	1 (0.9)	1 (1.6)	0	0
Anti-EJ	5 (2.1)	2 (1.8)	1 (1.6)	2 (3.4)	0
Anti–Mi-2	13 (5.4)	2 (1.8)	11 (17.7)	0	0
Anti-SRP	32 (13.2)	30 (27.0)	2 (3.2)	0	0
Other	4 (1.7)	4 (3.6)	0	0	0
Negative	129 (54.8)	44 (39.6)	33 (53.2)	43 (74.1)	5 (71.4)
MAAs					
All MAAs	94 (38.8)	38 (31.5)	14 (19.4)	40 (67.2)	2 (28.6)
Anti-PM/Scl	6 (2.5)	2 (1.8)	1 (1.6)	3 (5.2)	0
Anti-Ro	43 (17.8)	21 (18.9)	5 (8.1)	17 (29.3)	0
Anti-La	12 (5.0)	5 (4.5)	1 (1.6)	6 (10.3)	0
Anti-URNP	44 (18.2)	10 (9.0)	6 (9.7)	26 (44.8)	2 (28.6)
Anti-Ku	5 (2.1)	2 (1.8)	1 (1.6)	2 (3.4)	0
Other	6 (2.5)	3 (2.7)	2 (3.2)	1 (1.7)	0
Negative	154 (63.6)	76 (68.5)	50 (80.6)	19 (32.8)	5 (71.4)

* Of the total number of 262 African American patients (including 20 patients for whom the serologic data were unavailable), 201 (77%) were female and 61 (23%) were male. Serologic data were available for 111 of 115 patients with polymyositis (PM), 62 of 73 with dermatomyositis (DM), 58 of 63 with myositis/connective tissue disease (CTD) overlap, and all 7 with cancer-associated myositis (CAM). Other myositis-specific autoantibodies (MSA) comprised anti-KJ in 2 patients and anti-Ma in 2 patients. Other myositis-associated autoantibodies (MAAs) comprised anti-JP in 3 patients and anti-p155 in 3 patients. Anti-SRP = anti–signal recognition particle.

† MSAs and MAAs were not detected among the 5 patients with inclusion body myositis (IBM), and therefore results for the IIM subgroup of patients with IBM are not shown.

‡ The clinical subgroups of myositis/CTD overlap and CAM comprised 48 patients and 4 patients with PM, 14 patients and 2 patients with DM, and 0 patients and 1 patient with IBM, respectively; for 1 patient with myositis/CTD overlap, the clinical subgroup was not defined, and therefore the data were omitted.

autoantibody, and the frequency of patients coproducing any combination of myositis-specific and myositis-associated autoantibodies was ~14%. Anti-Ro and anti–Jo-1 autoantibodies were detected together in ~7% of African American IIM patients. Similar to that in European American IIM patients, the coincident presence of myositis-specific and myositis-associated autoantibodies was not observed among the anti–Mi-2 and anti-PM/Scl serogroups of African American IIM patients.

HLA associations with IIM clinicopathologic groups. HLA alleles found to be possible risk or protective factors ($P < 0.05$) or definite risk or protective factors (corrected P [P_{corr}] < 0.05, after correction for multiple comparisons) for different clinicopathologic groups of African American patients with IIM are summarized in Table 2. The HLA–A*6802 and DQA1*0501 alleles were identified as possible risk factors for IIM in African Americans. Moreover, DRB1*03 was also observed as a possible risk factor ($P = 0.011$, odds ratio [OR] 1.8, 95% confidence interval [95% CI] 1.13–2.78) when patients with IBM were excluded from our total number of African American IIM cases (data not shown).

Several HLA alleles (DRB1*0101, *0701, *1001, and DQA1*01) were identified as possible protective factors for IIM in African Americans. In addition, DRB1*14 was likewise a protective factor for IIM in African Americans, although the significance of this association is questionable given the unusually high frequency of DRB1*14 observed in our African American control population (~11% versus 1–4% reported previously [28,29]). The ethnic admixture of our African American control population, perhaps comprising some Latino and/or Native American populations (for whom DRB1*14 frequencies are ~12% and ~7%, respectively [28,30]), might partly explain these findings.

Whereas similar patterns of association were observed between African American patients with PM and the total group of African American IIM patients, a novel risk factor, DQA1*0601, was identified in African

Table 2. Summary of immunogenetic differences detected between different clinicopathologic groups of African American patients with IIM and unrelated, ethnically matched controls*

HLA allele	IIM (n = 262), no./total (%)†	Controls (n = 311) no./total (%)†	*P*	OR	95% CI
Total IIM (n = 262)					
HLA–A					
*6802	14/65 (21.5)	8/101 (7.9)	0.018	3.2	1.15–9.34
HLA–DRB1					
*0101‡	7/215 (3.3)	13/117 (11.1)	0.007	0.3	0.09–0.76
*0701‡	43/216 (19.9)	41/129 (31.8)	0.014	0.5	0.31–0.91
*1001‡	7/215 (3.3)	13/117 (11.1)	0.007	0.3	0.09–0.76
*14‡	7/229 (3.1)	23/204 (11.3)	0.014§	0.2	0.09–0.62
HLA–DQA1					
*0101‡	35/229 (15.3)	48/193 (15.9)	0.014	0.5	0.32–0.91
*0105‡	2/228 (0.9)	9/193 (4.7)	0.027	0.2	0.02–0.89
*0501	109/231 (47.2)	71/196 (36.2)	0.024	1.6	1.05–2.37
PM (n = 163)					
HLA–DRB1					
*0101‡	3/145 (2.1)	13/117 (11.1)	0.003	0.2	0.03–0.64
*0701‡	22/146 (15.1)	41/129 (31.8)	0.001	0.4	0.20–0.71
*1001‡	5/145 (3.4)	13/117 (11.1)	0.025	0.3	0.08–0.89
*14‡	5/150 (3.3)	23/204 (11.3)	0.008	0.3	0.08–0.76
HLA–DQA1					
*0501	70/144 (48.6)	71/196 (36.2)	0.026	1.7	1.05–2.64
DM (n = 87)					
HLA–DRB1					
*0301	21/66 (31.8)	12/113 (10.6)	0.026§	3.9	1.66–9.50
*14‡	2/74 (2.7)	23/204 (11.3)	0.031	0.2	0.02–0.93
HLA–DQA1					
*0101‡	11/82 (13.4)	48/193 (24.9)	0.037	0.5	0.21–0.99
*0601	5/82 (6.1)	1/193 (0.5)	0.010	12.5	1.35–592.6
Myositis/CTD overlap (n = 63)					
HLA–B					
*5301	7/11 (63.6)	23/94 (24.5)	0.012	5.4	1.22–27.0
HLA–DRB1					
*01‡	2/58 (3.4)	33/203 (16.3)	0.009	0.2	0.02–0.76
*0701‡	8/53 (15.1)	41/129 (31.8)	0.027	0.4	0.14–0.92
*08	16/59 (27.1)	19/203 (9.4)	0.013§	3.6	1.58–8.05

* Totals exclude patients with cancer-associated myositis. OR = odds ratio; 95% CI = confidence interval (see Table 1 for other definitions).
† Values are the number of allele-positive subjects/total number of subjects for whom complete low- or high-resolution HLA data were available at a given locus.
‡ Allele identified as a protective factor.
§ HLA alleles with corrected *P* values less than 0.05 (other alleles identified as probable disease susceptibility factors were defined as significant at $P < 0.05$, but when corrected, *P* values were greater than 0.05 and the 95% CI ranged from greater [risk] to less [protective] than 1.0).

American patients with DM. As described below, the detection of the DRB1*0301 risk factor for DM is likely attributable to the presence of anti–Jo-1 autoantibodies. A novel risk factor, DRB1*08, found commonly in linkage disequilibrium (LD) with HLA–B*5301, was identified in association with African American patients with myositis/CTD overlap, although the number of patients studied was small. The DRB1*08 allele group is also more frequently represented among African American patients with lupus, perhaps explaining, in part, the association with myositis overlap disease (31). Additional comparisons between juvenile and adult African American patients with IIM did not reveal any significant differences in HLA allele frequencies (data not shown). Consequently, juvenile and adult patients were combined in their respective clinicopathologic and serologic groups, to enhance the power of the study.

HLA associations with combined clinicopathologic and both myositis autoantibody groups. As summarized in Table 3, HLA alleles consistent with the 8.1 ancestral haplotype were identified as significant risk factors for IIM in African Americans expressing antisynthetase autoantibodies, in particular anti–Jo-1 autoantibodies. The DRB1*0301;DQA1*0501 haplotype was also a risk factor for DM in patients with anti-Ro autoantibodies, which is a myositis-associated autoanti-

Table 3. Summary of immunogenetic differences between different clinicopathologic groups of African American IIM patients with (MA+) or without (MA−) myositis autoantibodies and unrelated, ethnically matched controls*

Myositis autoantibody/ clinicopathologic group, HLA allele	IIM		Controls, no./total (%)	IIM MA+ vs. IIM MA−, P_{corr} (OR, 95% CI)	IIM MA+ vs. controls, P_{corr} (OR, 95% CI)†
	MA+, no./total (%)	MA−, no./total (%)			
Synthetase/all IIMs					
DRB1					
*03	27/60 (45.0)	32/108 (29.6)	47/205 (22.9)	NS	0.022 (2.8, 1.4–5.2)
*04	0/60 (0)	14/107 (13.1)	27/204 (13.2)	0.032 (ND)	0.015 (ND)
DQA1					
*0501	36/61 (59.0)	47/104 (45.2)	71/196 (36.2)	NS	0.026 (2.5, 1.4–4.8)
Jo-1/all IIMs					
HLA–B					
*08	8/16 (50.0)	3/26 (11.5)	13/112 (11.6)	NS	0.024 (7.6, 2.1–27.4)
DRB1					
*0301	16/36 (44.4)	16/100 (16.0)	12/113 (10.6)	0.039 (4.2, 1.6–10.6)	0.001 (6.7, 2.5–18.0)
Mi-2/all IIMs					
DRB1					
*0302	9/11 (81.8)	16/100 (16.0)	11/114 (9.6)	0.0005 (23.6, 4.2–234.2)	<0.0001 (42.1, 7.0–421.6)
DQA1					
*0401	10/11 (90.0)	29/102 (28.4)	36/193 (18.7)	0.0008 (25.2, 3.2–1,106)	<0.0001 (43.6, 5.7–1904)
*0501	0/11 (0.0)	47/104 (45.2)	71/196 (36.2)	0.024 (ND)	NS
SRP/all IIMs					
DQA1					
*0102	22/30 (73.3)	50/107 (46.7)	85/196 (43.4)	NS	0.040 (3.6, 1.4–9.8)
Ro/all IIMs					
DRB1					
*08	12/42 (28.6)	12/133 (9.0)	19/203 (9.4)	0.047 (4.0, 1.5–10.8)	0.025 (3.9, 1.5–9.4)
DQA1					
*0501	26/41 (63.4)	60/134 (44.8)	71/196 (36.2)	NS	0.021 (3.0, 1.4–6.6)
RNP/all IIMs					
DRB1					
*08	11/40 (27.5)	12/133 (9.0)	19/203 (9.4)	NS	0.047 (3.7, 1.4–9.1)
Jo-1/PM					
HLA–B					
*08	6/10 (60.0)	1/11 (9.1)	13/112 (11.6)	NS	0.027 (11.4, 2.3–60.7)
Synthetase/DM					
DRB1					
*0301	9/20 (45.0)	9/28 (32.1)	12/113 (10.6)	NS	0.024 (6.9, 2.0–22.5)
Jo-1/DM					
DRB1					
*0301	8/11 (72.7)	9/28 (32.1)	12/113 (10.6)	NS	0.0005 (22.4, 4.4–142.2)
DQA1					
*06	2/11 (18.2)	2/37 (5.4)	1/198 (0.5)	NS	0.044 (43.8, 2.0–2,594)
Mi-2/DM					
DRB1					
*0302	8/10 (80.0)	3/28 (10.7)	11/114 (9.6)	0.0034 (33.3, 3.6–400.1)	<0.0001 (37.4, 6.1–380.9)
DQA1					
*0401	10/11 (90.9)	6/36 (16.7)	36/193 (18.7)	0.0004 (45.0, 4.3–2,034)	<0.0001 (39.2, 5.0–1,731)
Ro/DM					
DRB1					
*0301	5/7 (71.4)	11/43 (25.6)	12/113 (10.6)	NS	0.020 (21.0, 2.9–231.8)
DQA1					
*0501	8/9 (88.9)	17/50 (34.0)	71/196 (36.2)	0.034 (15.5, 1.8–709.3)	0.031 (14.1, 1.8–629.8)
RNP/myositis/CTD overlap					
DRB1					
*08	9/25 (36.0)	2/16 (12.5)	19/203 (9.4)	NS	0.013 (5.4, 1.8–15.2)

* P_{corr} = P values (Fisher's exact test) corrected for multiple comparisons within each genetic locus; OR = odds ratio; 95% CI = 95% confidence interval; NS = not significant; ND = not determined (see Table 1 for other definitions).
† No significant differences ($P_{corr} < 0.05$) were detected between MA− and control comparison groups.

body found to be coexistent in ~12% of antisynthetase-positive myositis patients. In addition, novel risk factors (DQA1*06 in patients with DM) and protective risk factors (DRB1*04 in the total group of IIM patients) were identified among anti–Jo-1–positive and antisynthetase-positive African American patients, re-

spectively. In contrast, DRB1*0302 and DQA1*0401 (alleles commonly found in LD) were strong risk factors (OR $>$ 37) for IIM and DM among African American patients with anti–Mi-2 autoantibodies. Other novel findings included the identification of DQA1*0102 and DRB1*08 risk factors among African American IIM patients with anti-SRP and anti-RNP autoantibodies, respectively. No significant differences ($P_{corr} < 0.05$) in allele frequencies were detected between myositis-associated autoantibody–negative patients and controls, emphasizing the importance of serologic status in defining genetic risk.

Random Forests classification. Among the HLA alleles found in association with IIM in this study, it is unclear which factors may play a primary role in disease predisposition and which are associated secondarily or indirectly as the result of haplotype LD. To better define the status of individual HLA susceptibility factors in terms of their RI values (indicating their capacity to discriminate cases from controls), we utilized a Random Forests classification algorithm (as described in Patients and Methods). Random Forests modeling was performed for all HLA class II (DRB1 and DQA1) alleles identified among the myositis autoantibody subgroups anti–Jo-1 and anti–Mi-2 (data not shown). HLA alleles identified as susceptibility factors in univariate analyses were ranked by their RI in effectively classifying IIM cases and controls.

The DRB1*0301 allele ranked highest among all HLA class II alleles in African American patients with IIM and DM who were positive for anti–Jo-1 autoantibodies. In contrast, the structurally related DRB1*0302 allele ranked first among anti–Mi-2–positive African American IIM and DM patients. The closely linked HLA class II alleles DQA1*0501 and *0401 ranked lower than DRB1*0301 and *0302 alleles, respectively, confirming that the variant DRB1*03 alleles and/or a more closely linked gene or genes are the primary anti–Jo-1 and anti–Mi-2–associated risk factors. These Random Forests modeling data further demonstrated the strong risk conveyed by the anti–Mi-2–associated DRB1*0302 allele relative to DRB1*0701 in African Americans (the latter being the primary risk factor in European American anti–Mi-2–positive patients with DM) (12). Traditional logistic regression analyses independently corroborated these findings. For example, a comparative analysis of anti–Jo-1–positive African American IIM patients ranked HLA–DRB1*0301 (P = 0.0023, OR 8.2, 95% CI 2.1–31.6) highest among the HLA class II alleles that could discriminate IIM cases and controls.

HLA peptide-binding motifs among African American myositis autoantibody groups. Given the established importance of the HLA–DRB1 locus as a susceptibility factor for a host of human autoimmune diseases, including the IIMs, we examined multiple sequence motifs of primary amino acids (mapping within the HVR3 of the DRB1 gene) whose amino acid side chains make functional contacts within the fourth pocket of the MHC peptide-binding groove (HVR3 positions 70, 71, and 74). These RSP motifs were stratified according to consensus HVR3 amino acid sequences with established peptide and/or T cell receptor binding properties (32).

As expected, the RSP "R" motif ($Q^{\beta 70}/K^{\beta 71}/R^{\beta 74}$) representing the DRB1*03 HVR3 domain was a significant risk factor for IIM in patients producing antisynthetase and anti–Mi-2 autoantibodies (Table 4). Protective effects from RSP "A" were observed among patients producing antisynthetase autoantibodies. Interestingly, 3 DRB1 alleles defining part of the RSP "A" motif (DRB1*0101, *1001, and *1402) were also observed as protective factors both in the total group of IIM patients and in the patients with PM (see Table 2). In addition, a comparative analysis of DRB1 primary amino acid sequences among African American patients with DM for risk factors stratifying the anti–Jo-1 (DRB1*0301) and anti–Mi-2 (DRB1*0302 in African Americans and and *0701 in European Americans) autoantibody groups revealed an HVR2 amino acid motif (^{25}FLERYFHN^{32}) specific for the anti–Mi-2 phenotype (represented in 100% of African American IIM patients versus 36.6% and 39.7% of myositis-specific autoantibody–negative IIM patients and African American controls, respectively).

Comparisons of genetic factors between African Americans and European Americans. Comparisons of HLA allele associations between African American and European American patients with IIM revealed that different North American ethnic groups have, in some cases, shared immunogenetic susceptibility factors and in other cases have distinct immunogenetic susceptibility factors depending on the phenotype evaluated (Table 5). The 8.1 ancestral haplotype–derived DRB1*0301 risk factor was shared between African American and European American patients with DM and between African American and European American patients with anti–Jo-1 autoantibodies. Similarly, HLA–A*68, a definite risk factor for IIM in European Americans (9), was also identified as a potential risk factor for IIM in African Americans (see Table 2). In contrast to European Americans, DRB1*08 alleles were uniquely identified as risk factors for myositis/CTD overlap in African Amer-

Table 4. Summary of immunogenetic differences in DRB1 restrictive supertype pattern (RSP) functional motifs detected between African American IIM patients with (MA+) or without (MA−) myositis autoantibodies and unrelated, ethnically matched controls*

Autoantibody or DRB1, RSP pocket 4 motif (amino acids 70/71/74)†	IIM		Controls, no./total (%)‡	IIM MA− vs. IIM MA+, P_{corr} (OR, 95% CI)	IIM MA+ vs. controls, P_{corr} (OR, 95% CI)§
	MA+, no./total (%)‡	MA− no./total (%)‡			
Antisynthetase					
RSP "A" (QR/RK/A)¶	6/59 (10.2)	24/99 (24.2)	38/122 (31.1)	NS	0.012 (0.2, 0.08–0.66)
RSP "R" (Q/K/R)	27/60 (45.0)	32/108 (29.6)	47/205 (22.9)	NS	0.022 (2.8, 1.43–5.24)
Anti–Jo-1					
RSP "R" (Q/K/R)	21/37 (56.8)	32/108 (29.6)	47/205 (22.9)	NS	0.0013 (4.4, 2.00–9.78)
Mi-2					
RSP "R" (Q/K/R)	9/11 (81.8)	32/108 (29.6)	47/205 (22.9)	0.015 (10.7, 2.01–104.8)	0.0015 (15.1, 2.94–146.4)
DRB1 HVR2					
Mi-2					
^{25}FLERYFHN^{32}	11/11 (100.0)	37/101 (36.6)	52/131 (39.7)	0.0003 (ND)	0.0005 (ND)

* Totals exclude patients with cancer-associated myositis. P_{corr} = P values corrected for multiple comparisons; OR = odds ratio; 95% CI = 95% confidence interval; NS = not significant; HVR2 = second hypervariable region; ND = not determined (see Table 1 for other definitions).
† RSP defined as amino acid motifs occupying positions 70, 71, and 74 comprising pocket 4 of the HLA–DRB1 peptide-binding region.
‡ Values are the number of allele-positive subjects/total number of subjects for whom complete low- or high-resolution HLA data were available at a given locus.
§ No significant differences were detected between the MA− and control comparison groups, with the exception of the protective motif RSP "A" (P_{corr} = 0.047, OR 0.4, 95% CI 0.22–0.82).
¶ RSP motif identified as a potential protective factor for IIM.

icans and in the anti-Ro/anti-RNP autoantibody groups. Clear distinctions were also observed between African Americans and European Americans regarding HLA risk factors for anti-SRP and anti–Mi-2 autoantibodies. In the latter case, although DRB1*0701 is a primary risk factor for anti–Mi-2 autoantibodies in European Americans and is prevalent in African American populations, DRB1*0302 was found to be the predominant risk factor in African Americans with these autoantibodies.

A recent large study of myositis–HLA associations among European Caucasian patients in the United Kingdom (33) yielded results similar to those in our European American studies, thus further substantiating the genetic differences between myositis populations of European and African descent. Together, these data suggest that in addition to shared HLA susceptibility factors with European American patients with IIM, different clinical and serologic groups of African Amer-

Table 5. Summary comparison of primary HLA susceptibility factors in African American and European American patients with IIM*

	HLA allele association	
	African Americans	European Americans†
Total IIM	DRB1*14‡	B*0801, DRB1*0301
PM	None	B*0801, DRB1*0301
DM	DRB1*0301	B*0801, DRB1*0301
Myositis/CTD overlap	DRB1*08	B*0801, DRB1*0301
Synthetase	DRB1*03, *04, RSP "A"‡	B*0801, DRB1*0301, RSP "A"‡
Jo-1	DRB1*0301	B*0801, DRB1*0301
Mi-2	DRB1*0302, ^{25}**FLE**RYFHN^{32}§	DRB1*0701
SRP	DQA1*0102	B*5001
Ro	DRB1*08	B*0801, DRB1*0301
RNP	DRB1*08	None

* Reported alleles represent factors significant after correction for multiple comparisons and having high relative importance scores in Random Forests analyses. RSP = restrictive supertype pattern (see Table 1 for other definitions).
† Complete lists of all IIM-associated European American alleles were reported previously (9,12).
‡ Identified as a protective factor for IIM.
§ In the DRB1 second hypervariable region, amino acids (shown in boldface type) are shared between the DRB1*0302 and *0701 alleles but are distinct from the DRB1*0301 allele.

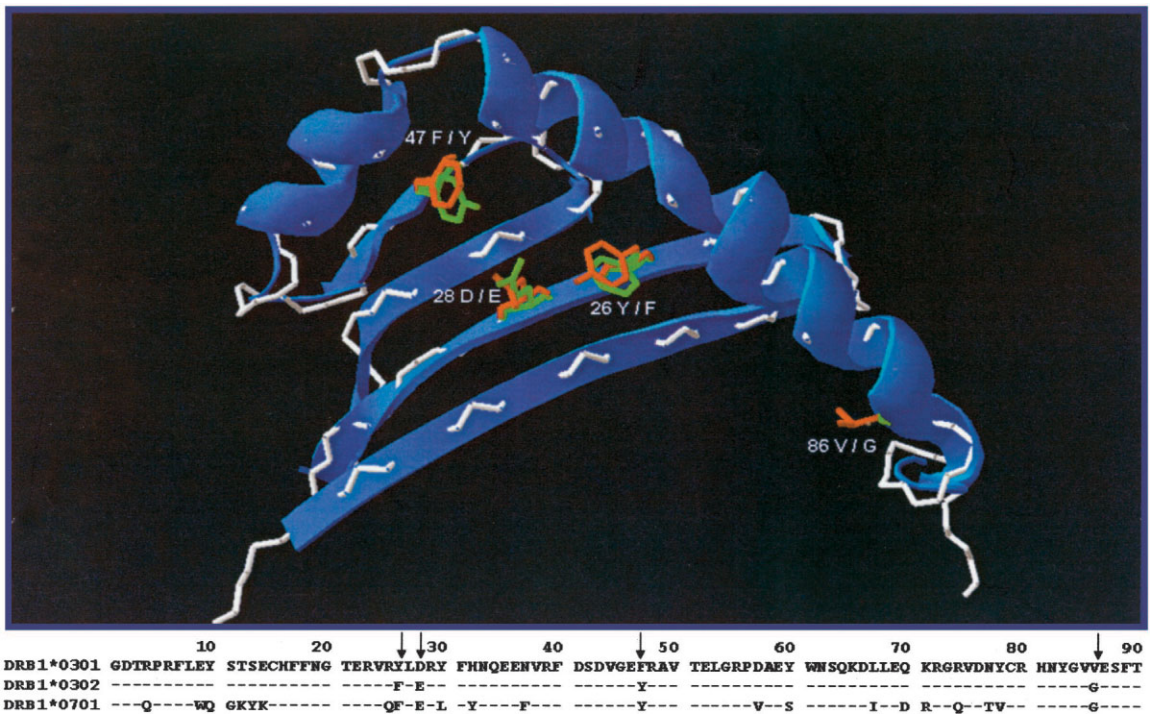

Figure 1. Three-dimensional comparative homology modeling of anti–Mi-2– and anti–Jo-1–associated HLA risk factors in African American and European American patients with idiopathic inflammatory myopathy (IIM). The comparative protein modeling of HLA risk factors for IIM in patients producing anti–Jo-1 (DRB1*0301 in African Americans and European Americans) and anti–Mi-2 (DRB1*0302 in African Americans and *0701 in European Americans) was performed using the SWISS-MODEL server and DEEPVIEW Swiss-Pdb Viewer software as described in Patients and Methods. The resulting DRB1 models were structurally aligned and optimized within SWISS-MODEL to produce a final composite 3-dimensional structure of the DRB1*0301, *0302, and *0701 peptide-binding region (ribbon diagram in blue and α-carbon backbone in white). Side chains of amino acids conserved between DRB1*0302 and *0701 (green) and divergent from DRB1*0301 (red) (Y/F$^{\beta 26}$, D/E$^{\beta 28}$, F/Y$^{\beta 47}$, and V/G$^{\beta 86}$) are also shown.

ican patients with IIM have unique immunogenetic features, perhaps consistent with alternative pathways of disease development.

Molecular modeling of anti–Jo-1– and anti–Mi-2–associated risk factors in African Americans and European Americans. Our laboratory and other investigators have established DRB1*0301 as an important risk factor for IIM in European Americans producing anti–Jo-1 autoantibodies (12). In the present study, we likewise identified DRB1*0301 as a significant and primary risk factor for anti–Jo-1 in IIM and in DM among African Americans. Among European American patients with IIM and those with DM, DRB1*0701 is the primary anti–Mi-2–associated risk factor (12). In contrast, the DRB1*0302 allele appears to be associated with IIM and DM in African American patients who have anti–Mi-2 autoantibodies, despite higher frequencies of DRB1*0701 in our African American control population. These differences may be explained, in part, by the near absence of the DRB1*0302 allele in most European American populations (34,35).

Together, these data suggest that certain amino acids conserved between DRB1*0302 and DRB1*0701 (amino acids F$^{\beta 26}$, E$^{\beta 28}$, Y$^{\beta 47}$, and G$^{\beta 86}$) may contribute to the preferential binding of an immunodominant Mi-2 autoantigenic peptide. These observations are striking, considering that anti–Jo-1– and anti–Mi-2–associated risk factors in African Americans (DRB1*0301 and DRB1*0302, respectively) differ by only 4 amino acid residues within the exon 2–encoded peptide-binding domain (Y/F$^{\beta 26}$, D/E$^{\beta 28}$, F/Y$^{\beta 47}$, and V/G$^{\beta 86}$). We hypothesized that amino acids conserved between anti–Mi-2–associated risk factors DRB1*0302 and *0701 might share similar 3-D orientations within the peptide-binding groove of the mature DRB1 molecule. To address this possibility, we utilized a comparative homology approach to model the peptide-binding groove of the DRB1*0301, *0302, and *0701 molecules (as described in Patients and Methods).

As shown in Figure 1, a composite alignment of the overlapping structures confirmed that amino acid side chains at polymorphic positions distinguishing the

DRB1*0301 and *0302 (positions β26, β28, β47, and β86) were oriented identically between the DRB1*0701 and *0302 molecules. The net result of these amino acid variations between DRB1*0301 and *0302/*0701 is the alternative placement of a hydroxyl group between positions β26 and β47 and a modest variation in the side-chain length of the carboxylic acid group (position β28) along the β-sheet base formation of the peptide-binding groove. In addition, a short aliphatic side chain was introduced at position β86 of the α-helical coil comprising the wall of the peptide-binding domain. Amino acids at each of these positions have been previously documented to make direct contacts with bound peptides and/or cognate T cell receptor molecules, which establishes, in part, the peptide-binding characteristics of the respective alleles (36).

DISCUSSION

The present study is the largest study conducted to date of HLA class I and class II allelic associations in a North American minority population with IIM. We were able to identify multiple, novel genetic risk and protective factors for different clinicopathologic and myositis autoantibody groups. Prior genetic studies of autoimmune disease among African Americans have largely focused on rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and type 1 diabetes mellitus (T1D) (37–39). Similar studies of rarer conditions in minority populations, including IIM, are generally few and often statistically underpowered.

We identified alleles associated with the 8.1 ancestral haplotype, HLA–B*08 and DRB1*0301, as significant risk factors for the development of IIM in African American patients producing anti–Jo-1 autoantibodies. Previous studies that failed to demonstrate this association were likely constrained by smaller sample sizes or lack of autoantibody stratification (13,15). Associations with the DRB1*0301 allele have also been described among African American patients with SLE and T1D, perhaps in keeping with the more generalized immune dysregulatory properties attributed to the 8.1 ancestral haplotype (40,41). The association of the HLA–B*08;DRB1*0301 haplotype with IIM in African Americans may be, in part, a consequence of genetic admixture; the European American–derived 8.1 ancestral haplotype is a predominant haplotype in both European American and African American populations (34,42). A recent examination of SLE patients derived from the LUpus in MInorities, NAture versus nurture (LUMINA) study group (147 European Americans and 181 African Americans) estimated that ~20% of the genome in African Americans is of European American extraction (43). The effects of genetic admixtures in augmenting disease susceptibility have been proposed to explain the higher prevalence of RA among African Americans compared with native Africans (37).

We also identified several novel HLA risk factors, including DRB1*08, which was detected at increased frequencies among African American patients with myositis overlap and those producing anti-RNP and anti-Ro autoantibodies. Increased frequencies of DRB1*08 alleles have also been described among African American patients with SLE within the LUMINA study group (31). In contrast to the anti–Jo-1–positive serogroup, HLA allelic associations among other African American myositis-specific autoantibody–positive groups appear distinct from their European American counterparts. Differences in anti–Mi-2–associated risk factors between European American and African American patients (DRB1*0701 and *0302, respectively) are of particular interest considering the prevalence of the DRB1*0701 and *0302 alleles in our African American control population (~21% and ~10%, respectively) and the near absence of the DRB1*0302 allele among our and other European American control populations (35). Moreover, use of a Random Forests prediction and classification algorithm consistently ranked DRB1*0301 and *0302 highest among all DRB1 alleles in distinguishing African American controls and African American IIM patients producing anti–Jo-1 and anti–Mi-2 autoantibodies, respectively. Based on these findings, we propose a consensus amino acid sequence motif in HVR2 of DRB1, ^{25}FLERYFHN^{32}, corresponding to conserved elements of the transethnic, anti–Mi-2–associated risk factors (DRB1*0302 and *0701).

We hypothesized that risk factors for anti–Mi-2 autoantibodies in European Americans and African Americans might encode amino acids with similar orientations within the MHC peptide-binding region. Superimposed 3-D homology models encompassing shared allelic polymorphisms of DRB1*0701 and *0302 (amino acids F$^{\beta 26}$, E$^{\beta 28}$, Y$^{\beta 47}$, and G$^{\beta 86}$) were used to predict that their respective amino acid side chains are oriented identically within the peptide-binding groove. In contrast, structural variations of amino acid side chains at corresponding positions were predicted for the anti–Jo-1–associated risk factor, DRB1*0301. These data suggest that similarities between the 3-D structures of DRB1*0701 and *0302, yet distinct from DRB1*0301, might influence the differential binding of Jo-1– and Mi-2–derived autoepitopes. Transethnic variations in the selection of anti–Mi-2–associated risk factors may

represent a hierarchical relationship between DRB1*0302 and *0701, resulting from differential affinity and/or avidity for a common autoantigenic peptide. The usefulness of such comparative homology modeling was demonstrated recently in a study by Ettinger et al, in which functional correlates were observed for structurally similar susceptibility factors for T1D (DQB1*0602 and *0604) (44).

There are several limitations to our case–control, candidate gene study design, including diminished statistical power when comparing smaller subsets of patients, and incomplete data for all HLA loci in the total subject population. Moreover, variability attributed to different genetic admixtures in different geographic locations among North American ethnic groups may skew the allele frequencies observed among some immune response genes (29,43). We attempted to mitigate some of these effects, in part, by selecting comparable numbers of ethnically and geographically matched controls from the referral centers. Despite these limitations, our data demonstrate convincingly that HLA alleles are markers for different clinical and serologic (i.e., myositis autoantibody) phenotypes among African American patients with IIM, which is consistent with the hypothesis that divergent pathogenic mechanisms can partially account for the heterogeneity of the disease. These data are also consistent with the findings from studies of other autoimmune disorders that have identified different genetic risk factors for varying phenotypes within a given disease (1,45–47).

In summary, the detection of shared and distinctive HLA susceptibility factors for IIM among African Americans and other ethnogeographic groups further exemplifies the complex polygenic and multifactorial nature of disease susceptibility (6). It is likely that genetic and environmental risk and protective factors influence the development of IIM, not only among ethnic groups, but also within subgroups of patients displaying particular clinical and serologic phenotypes (9,12,48–51). To illustrate this point, our present study identified a number of novel genetic risk factors associated with varying phenotypes of IIM in African Americans, and presented the putative definition of an amino acid motif contributing to the selection of Mi-2–derived autoantigenic peptides, a model that can now be tested in future functional studies.

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