

Modification of Multiple Sclerosis Phenotypes by African Ancestry at HLA

Bruce A. C. Cree, MD, PhD, MCR; David E. Reich, PhD; Omar Khan, MD; Philip L. De Jager, MD, PhD; Ichiro Nakashima, MD; Toshiyuki Takahashi, MD; Amit Bar-Or, MD; Christine Tong, BS; Stephen L. Hauser, MD; Jorge R. Oksenberg, PhD

Background: In those with multiple sclerosis (MS), African American individuals have a more severe disease course, an older age at onset, and more often have clinical manifestations restricted to the optic nerves and spinal cord (opticospinal MS) than white persons.

Objective: To determine whether genetic variation influences clinical MS patterns.

Design: Retrospective multicenter cohort study.

Participants: Six hundred seventy-three African American and 717 white patients with MS.

Main Outcome Measures: Patients with MS were genotyped for *HLA-DRB1* and *HLA-DQB1* alleles. The proportion of European ancestry at HLA was estimated by genotyping single-nucleotide polymorphisms with known significant frequency differences in West African and European populations. These genotypes were correlated with

the opticospinal disease phenotype, disability measures, and age at onset.

Results: Subjects with *DRB1*15* alleles were twice as likely to have typical MS rather than opticospinal MS ($P = .001$). Of the subjects with opticospinal MS or a history of recurrent transverse myelitis who were seropositive for anti-aquaporin 4 antibodies (approximately 5%), none carried *DRB1*15* alleles ($P = .008$). Independently of *DRB1*15*, African ancestry at HLA correlated with disability as measured by the Multiple Sclerosis Severity Score ($P < .001$) and risk of cane dependency (hazard ratio, 1.36; $P < .001$); *DRB1*15* alleles were associated with a 2.1-year earlier age at onset ($P < .001$).

Conclusions: These data indicate that the role of HLA in MS is not limited to disease susceptibility but that genes embedded in this locus also influence clinical outcomes.

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COMPARED WITH WHITE individuals, African Americans with multiple sclerosis (MS) more often have symptoms restricted to the spinal cord and optic nerves (opticospinal MS) and are at a higher risk of ambulatory disability.¹⁻⁶ In white people, these traits are believed to be partially genetically based.⁷ Genetic factors play an important role in determining susceptibility for MS and perhaps influence clinical outcomes.⁷⁻¹² The HLA region is unequivocally the most important MS susceptibility locus genome wide,^{13,14} with a primary signal arising from *DRB1* (OMIM 142857) (specifically *DRB1*15* alleles) in the class II region.¹⁵ To better understand how the HLA locus influences MS phenotypes, we sought to correlate HLA genotypes with MS phenotypes in a large data set of African American and white patients with MS.

Author Affiliations are listed at the end of this article.

METHODS

COLLECTION OF DATA SETS

Medical records and blood were collected from self-identified African American and white patients with MS through a nationwide network of MS centers.¹⁵ Written informed consent was obtained from all participants and the study received institutional review board approval. Multiple sclerosis phenotypes were characterized by systematic medical record review,³ and all subjects met International Panel of MS Diagnosis criteria for the disease.¹⁶ Opticospinal MS was defined by a history of relapses and clinical signs restricted to the optic nerves and spinal cord. A minimum of 5 years of follow-up after onset of the first demyelinating event was required for subjects to be considered to manifest symptoms in other anatomic areas. Transverse myelitis was defined as bilateral paresis with alteration in somatosensation corresponding to a spinal cord level and sphincter impairment. Cases of paraparesis and paraplegia were included even if the sensory level was incom-

plete. Disability was measured using the Multiple Sclerosis Severity Score (MSSS)¹⁷ and the Expanded Disability Status Scale (EDSS).¹⁸

Subjects whose records were incomplete, did not confirm a diagnosis of clinically definite MS (25 African American subjects), or were consistent with other diagnoses (15 African American and 2 white subjects) were excluded. The final data set consisted of 673 African American and 717 white subjects. Based on the 2000 census,¹⁹ the risk of MS in the white population,²⁰ and the relative risk of MS in African Americans,^{21,22} our cohort of 673 patients is estimated to be a 3.6% to 5.4% sample of African Americans with MS in the United States. African American and white subjects were recruited from 39 and 44 states, respectively. Unaffected control subjects, including spouses and friends of the patients, were also enrolled.

HLA GENOTYPING AND ADMIXTURE MAPPING AT THE 6P21 REGION

Genotyping for *HLA-DRB1* and *HLA-DQB1* alleles was performed as previously described.¹⁵ Individual ancestry estimates for the HLA locus were determined using the admixture mapping method previously described.^{23,24} In brief, this technique uses single-nucleotide polymorphisms (SNPs) that are highly informative for either European or African ancestry to determine individual ancestry estimates expressed as the percentage of admixed European ancestry for each individual. In addition to being able to provide an estimate of global ancestry, the ANCESTRYMAP software can be used to obtain local estimates of the proportion of European to African ancestry at each position in the genome for each individual. In this study, the marker rs3135391,²⁵ which is associated with the DRB1*1501 allele, was used to define the position of the HLA locus at which to center the local ancestry estimate. Data from all the markers genotyped on chromosome 6 surrounding HLA (61 in all) were used to estimate each individual's ancestry. When expressed as the extent of African ancestry, this number varies between 0 and 2, where 0 reflects 100% European origin for both chromosomes and 2 represents 100% African origin for both chromosomes at the specified region of interest. The farther away an SNP is in genetic distance, the higher its probability of having become unlinked from HLA as a result of a recombination event since European-African admixture began and the less information it provides about ancestry. Therefore, SNPs close to DRB1*1501 were weighted more heavily than SNPs farther away.²³

AQUAPORIN-4 IMMUNOREACTIVITY

This study recruited subjects with opticospinal MS prior to the development of the neuromyelitis optica (NMO)-IgG antibody assay.²⁶ This autoantibody is directed against the water channel aquaporin 4 (AQP4)²⁷ and distinguishes NMO from MS with 91% sensitivity and 100% specificity.^{28,29} Some of the subjects with opticospinal MS in this cohort could be seropositive for the anti-AQP4 antibody and therefore would meet new diagnostic criteria for NMO.³⁰ All subjects with either opticospinal MS (n=125) or recurrent transverse myelitis (n=74) were assessed for anti-AQP4 seropositivity.

In brief, AQP4-transfected human embryonic kidney cells were incubated with diluted serum, washed, and incubated with fluorescein-conjugated goat anti-human IgG. Fixed cells were photographed with confocal microscopy, and antibody seropositivity was scored through comparison with transfected cells that did not express AQP4. Assays were performed as previously described,^{28,29} except the serum samples were diluted 1:16 in phosphate-buffered saline and the detection antibody was changed to Alexa Fluor 488 goat anti-human IgG (Invitrogen, Eugene, Oregon). The serum dilution was changed from the

published 1:4 dilution because of a high background signal in some samples at this dilution. In addition, all previously reported positive samples were positive at dilutions greater than 1:16. Therefore, screening at 1:16 does not influence the sensitivity of this assay and reduces background fluorescence.

We used Stata, version 9 (Stata Corp, College Station, Texas), for statistical analyses. Categorical variables were compared using the χ^2 test. Continuous variables were compared using the *t* test or Wilcoxon test, as appropriate. Multivariate models initially included all baseline covariates. Clinical variables that were initially included in the models were race, age at disease onset, sex, anatomical location of onset, sensory vs motor onset, opticospinal vs classic/multifocal disease, optic neuritis, and transverse myelitis occurring at anytime during the course of the disease. Variables that did not significantly contribute to the model were removed stepwise until a final model was reached in which all variables retained statistical significance. Tests for interactions were performed but no interactions were found. Subjects who were seropositive for the anti-AQP4 antibody were excluded from the cohort for the phenotype-genotype regression analyses.

Survival analysis was used to assess time-dependent variables. The disease duration was defined as the time from disease onset to the last documented neurological examination. Time to ambulation with a cane was defined as time from disease onset to the time when the subject was unable to ambulate without unilateral assistance (EDSS score=6). Kaplan-Meier curves were used to explore and describe survivor functions across groups and were compared using the log-rank test for equality of survivor functions. The Cox proportional hazard model was used to assess the effect of predictors of event times using the likelihood ratio test.

To control for differences in type and duration of treatment between the 2 racial groups, time-dependent covariates for both US Food and Drug Administration–approved and non–Food and Drug Administration–approved therapies that are commonly used to treat MS were constructed. Time-dependent covariates account for the actual periods during which therapy was received, changes in the class of therapy, and intervals during which subjects were not taking disease-modifying treatments. Four treatment groups were defined: interferon beta, glatiramer acetate, cytotoxic, and other. The interferon beta category included treatment with any of the interferon preparations (interferon beta-1b and the 2 interferon beta-1a products). Similarly, the cytotoxic group included any cytotoxic agent (eg, mitoxantrone, cyclophosphamide, azathioprine, methotrexate, or cladribine). The other category included monthly pulse-dosed glucocorticoids, intravenous immunoglobulin, and experimental protocols. Treatments that were administered to patients for less than 2 months were not considered to be of sufficient duration to alter the course of the disease and were not included.

RESULTS

CLINICAL FEATURES OF AFRICAN AMERICAN AND WHITE PATIENTS

The clinical characteristics of the cohort are summarized in **Table 1**. African Americans had an older age at onset, experienced greater disability, were at increased risk for secondary progressive MS, experienced transverse myelitis more often, were more likely to have motor symptoms at onset, and were more likely to have the opticospinal disease subtype. These substantial differences in disease expression between African American and white patients afford the opportunity to investigate the role of genetic factors in driving these MS phenotypes.

Table 1. Demographic and Clinical Characteristics of a Cohort of Patients With MS

Clinical Characteristic	Patients, No. (%)		P Value
	White (n=717)	African American (n=673)	
Sex, F/M, No.	556/161	539/134	.12
Age at onset, mean (SD), y	29.9 (8.6)	32.8 (9.7)	<.001
Disease duration, mean (SD), y	9.9 (8.4)	9.7 (7.8)	.62
Disease type			
RRMS	71.6 (513)	59.7 (402)	.001
SPMS	155 (21.6)	182 (27.0)	
PPMS	33 (4.6)	46 (6.8)	
PRMS	6 (1.5)	24 (1.8)	
Unknown course ^a	5 (0.7)	17 (4.6)	
Time from disease onset to diagnosis, median (mean), y	1 (3.4)	1 (3.1)	.33
MSSS, mean (SD)	4.47 (2.6)	5.6 (2.8)	<.001
HR for time to cane dependency ^b		1.96	<.001
Opticospinal MS	53 (7.4)	72 (11.0)	.02
Transverse myelitis	84 (11.7)	180 (27.0)	<.001
Motor onset	138 (19.2)	209 (31.0)	<.001
Seropositive for anti-aquaporin 4	3 (4.2)	8 (6.2)	.55

Abbreviations: HR, hazard ratio; MS, multiple sclerosis; MSSS, Multiple Sclerosis Severity Score; PPMS, primary progressive multiple sclerosis; PRMS, progressive relapsing multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.

^aPatients could not be classified into 1 of the preceding categories.

^bExpanded Disability Status Scale score=6.

The proportion of subjects with relapsing-remitting MS vs secondary progressive MS in African American and white patients remained statistically significant after Bonferroni correction ($P=.005$). No other comparisons of disease course with ethnicity retained statistical significance. African Americans have a more disabling disease with a higher average MSSS compared with white individuals. Survival analysis was used to further explore the influence of ethnicity on the major disability milestone: the time at which individuals require a cane to ambulate (EDSS score=6). The hazard ratio shows that African Americans have an approximately 2-fold increased risk of requiring a cane to ambulate relative to white patients with MS. Anti-aquaporin 4 seropositivity was assessed in the subset of patients who had either opticospinal MS or recurrent transverse myelitis (71 white and 128 African American patients). A sensitivity analysis excluding all subjects with progressive disease found similar results with regard to disability, age at onset, and motor symptoms at onset.

Differences in socioeconomic status affect access to health care and could confound an analysis of disease severity. Adjustments for differences in socioeconomic status were not made for this cohort because this information was not available; however, access to health care was investigated through several surrogate indices.³ First, all patients received care through neurological clinics. Second, the time from disease onset to diagnosis was similar for African American and white patients. This is a surrogate marker for access to health care because lack of access to care would potentially delay the time from disease onset to diagnosis. Third, the time from diagnosis to initiation of disease-modifying therapies was the same

between these groups. Fourth, the type and duration of treatment was rigorously controlled for using time-dependent covariates in the survival analysis; no significant differences were found between the 2 groups. Thus, both African American and white patients received similar care in terms of receiving a timely diagnosis and disease-modifying treatment. Nevertheless, the analysis of disease severity may be confounded by the consequences of other presumed differences in socioeconomic status, ascertainment bias, or other environmental factors for which we were unable to control.

HLA-DRB1*1501 AND HLA-DRB1*1503 HAPLOTYPES

More than half of white patients (53.3%) had at least 1 copy of the DRB1*15 haplotype. The corresponding frequency in unaffected white controls was 16.0%. In contrast, 37.6% of African Americans with MS had at least 1 copy of DRB1*15; 10.3% of African Americans carried DRB1*1501, and 27.3% carried DRB1*1503, the uniquely African MS susceptibility allele.¹⁵ In unaffected African American controls, the corresponding frequency for DRB1*15 alleles was 14.7% (4.4% for DRB1*1501 and 10.3% for DRB1*1503). These results confirm that African American and white patients with MS are enriched for DRB1*15 haplotypes and that both DRB1*1501 and DRB1*1503 alleles contribute to this association in African Americans.

HLA CORRELATIONS WITH OPTICOSPINAL MS

The influence of DRB1*15 (both DRB1*1501 and DRB1*1503) alleles on the classic/multifocal disease course compared with the opticospinal disease subtype was explored. Opticospinal MS is defined by signs and symptoms of MS restricted to the optic nerves and spinal cord in patients with the disease for at least 5 years.³ Patients with opticospinal MS may have brain lesions on magnetic resonance imaging that are consistent with MS¹⁶ and usually do not have multisegmental spinal cord lesions that are typical of NMO. Patients who clearly met clinical diagnostic criteria for typical NMO were excluded.³¹ However, because of the inherent limitations in acquiring data through a multicenter medical record review, we were unable to ascertain the length of spinal cord lesions for every attack of myelitis.

Subjects with DRB1*15 alleles were more likely to manifest the classic/multifocal disease course rather than the opticospinal subtype (**Table 2**). Multivariate regression modeling did not identify other covariates associated with this distinction. After adjusting for DRB1*15 alleles, African ethnicity was not independently associated with opticospinal MS. Thus, the observation that African American individuals with MS more often manifest the opticospinal disease phenotype is driven by their relatively lower prevalence of DRB1*15 alleles compared with white individuals. The **Figure** further illustrates this association: 38.7% of African American patients who had a typical disease type carried DRB1*15. In contrast, only 20.8% of African American patients with an opticospinal disease type carried DRB1*15, which is not significantly

Table 2. Genetic and Phenotypic Characteristics Influencing the Risk for Developing Opticospinal MS in 125 Subjects^a

Characteristic	OR (95% CI)	P Value
Unadjusted analysis		
DRB1*15 (n=541)	0.52 (0.35-0.77)	.001
African American ethnicity (n=673)	1.46 (1.01-2.12)	.047
Adjusted analysis		
DRB1*15 (n=541)	0.54 (0.37-0.80)	.003
African American ethnicity (n=673)	1.32 (0.91-1.94)	.14

Abbreviations: CI, confidence interval; MS, multiple sclerosis; OR, odds ratio.

^aFifty-nine African American and 92 white subjects could not be assigned to either the opticospinal or typical disease type, either because their disease duration was shorter than 5 years or because age at onset could not be determined.

different from the healthy African American control population frequency of 14.7%. Thus, in African Americans, classic/multifocal MS is associated with DRB1*15 alleles, whereas opticospinal MS is not. Trends for a dosage effect of DRB1*15 were also observed in these phenotypes in African Americans (14.9% of those negative for DRB1*15, 7.1% of DRB1*15 heterozygotes, and 4.2% of DRB1*15 homozygotes have opticospinal MS).

Of the 199 subjects with opticospinal MS or recurrent transverse myelitis who were assessed for the anti-AQP4 antibody, 11 were seropositive: 9 subjects with opticospinal MS and 2 subjects with recurrent transverse myelitis. Three of 71 white patients and 8 of 128 African American patients tested were seropositive for AQP4 ($P = .75$, 2-sided Fisher exact test). Interestingly, 0 of the 11 subjects seropositive for anti-AQP4 carried the DRB1*15 allele ($P = .008$, 2-sided Fisher exact test), suggesting that anti-AQP4 seropositive subjects may be immunogenetically different from those with typical MS. Given that only 11 of the 199 subjects with opticospinal MS or recurrent transverse myelitis were seropositive for AQP4, this study is underpowered to draw conclusions about possible HLA associations with NMO susceptibility. Nevertheless, that all 11 subjects seropositive for anti-AQP4 are negative for DRB1*15 suggests that DRB1 alleles may influence NMO susceptibility and deserve further study in a larger cohort. The identification of a subgroup of patients with MS who were found to be AQP4-seropositive illustrates the difficulty in distinguishing NMO from MS using clinical criteria without anti-AQP4 antibody testing.

ADMIXTURE MAPPING AT 6P21

Correlation With HLA

Individual estimates for the proportion of African ancestry at HLA were determined for each DRB1 haplotype of interest (Table 3). For the African American MS population as a whole, African ancestry at HLA was 1.56 chromosomes, corresponding to an estimated 78% African and 22% northern European ancestry, identical to our previously reported genome-wide estimate.²⁴

As expected, DRB1*1503 haplotypes show the highest levels of African ancestry (1.16-1.75, range includes DRB1*1501/DRB1*1503 heterozygotes; heterozygosity

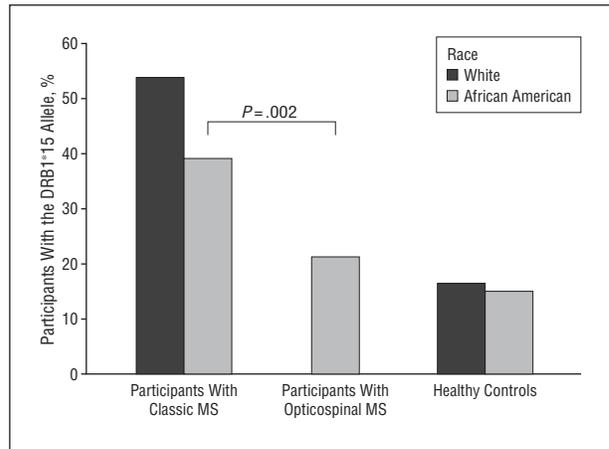


Figure. The DRB1*15 allele frequency in patients with classic multiple sclerosis (MS) or opticospinal MS and healthy controls. In African Americans, DRB1*15 alleles are more often associated with a classic/multifocal rather than an opticospinal disease type.

dilutes the effect of the DRB1*1503 haplotype). DRB1*1501-DQB1*X haplotypes have an intermediate ancestry value of 0.73, whereas non-DRB1*15 haplotypes show high African ancestry estimates of 1.60 with a broad range (0.01-2.00). Interestingly, the ancestry estimates for at least some of the subjects with the typical northern European DRB1*1501-DQB1*0602 haplotype appeared to be of African origin (mean African ancestry of 0.96 and 0.50 for heterozygotes and homozygotes, respectively). These observations are consistent with the hypothesis that not only the DRB1*1501 allele but also the extended DRB1*1501-DQB1*0602 haplotype commonly found in northern European populations are in some cases present on chromosomal segments that are African in origin rather than being due to admixture.

Correlation With Disease Severity and African Ancestry at HLA

Using linear regression, a correlation was observed between the degree of African ancestry at HLA and extent of MS disability measured by MSSS (Table 4). The MSSS is an effective tool for comparing disease progression using cross-sectional evaluation of MS-related disability, taking into account disease duration.¹⁷ As with the EDSS,¹⁸ severity increases as the MSSS increases between 0 and 10. Multivariate modeling found additional significant contributions to the MSSS for age at onset, male sex, motor onset, spinal cord onset, and a history of recurrent transverse myelitis. After adjusting for these other factors, African origin at HLA accounts for 0.56 points of the MSSS, or 10% of the mean MSSS score in African Americans (mean MSSS, 5.6; standard deviation, 2.8) (Table 1). Thus, African origin at HLA accounts for 50.9% of the overall 1.10-point difference (95% confidence interval, 35.5%-67.3%) in the mean MSSS between African American (MSSS, 5.55) and white (MSSS, 4.45) patients. These observations suggest that the difference in disability between African American and white patients is determined to a significant extent by allelic differences in a gene or genes within the HLA locus. Importantly, the addition of either DRB1*1501 or

Table 3. African Ancestry Estimates for HLA in Patients With Multiple Sclerosis

<i>DRB1-DQB1</i> Haplotype ^a	No. of Patients	Ancestry Estimate ^b		
		Mean (SD)	Minimum	Maximum
X-X/X-X	423	1.60 (0.49)	0.01	2.00
1501-0602/X-X	45	0.96 (0.42)	0.03	1.89
1501-0602/1501-0602	2	0.50 (0.66)	0.03	1.00
1501-0602/1501-X	1	0.03	0.03	0.03
1503/X	166	1.75 (0.36)	0.45	2.00
1503/1503	8	1.74 (0.26)	1.34	2.00
1501-0602/1503-X	10	1.16 (0.44)	0.42	1.99
1501-unknown/X-unknown ^c	18	0.73 (0.45)	0.11	1.47

^aX represents any other allele.

^bThe ancestry estimate is for both chromosomes. The minimum value of 0.00 corresponds to 100% Northern European origin, and the maximum value of 2.00 corresponds to 100% African origin. The *HLA-DRB1* and *HLA-DQB1* genotypes were determined as previously described.¹⁵

^cFor 18 subjects *DQB1* genotyping was not determined.

Table 4. Genetic and Phenotypic Influences on Disability as Measured by the MSSS

Analysis	Coefficient (95% CI)	P Value
Unadjusted analysis, African origin at HLA ^a	0.67 (0.50-0.84)	<.001
Adjusted analysis ^b		
African origin at HLA	0.56 (0.39-0.74)	<.001
Male sex (n=294)	0.55 (0.21-0.89)	.002
Age at onset, per year	0.06 (0.04-0.08)	<.001
Brainstem/cerebellar presentation (n=169)	0.94 (0.41-1.47)	.001
Recurrent transverse myelitis (n=87) ^c	0.86 (0.27-1.45)	.004
Motor symptoms at onset (n=342)	1.02 (0.66-1.37)	<.001

Abbreviations: CI, confidence interval; MSSS, Multiple Sclerosis Severity Score.

^aSubjects seropositive for anti-aquaporin 4 were excluded, the primary consequence of which was a reduction in the magnitude of the coefficient for recurrent transverse myelitis (data not shown).

^bCovariates that did not significantly contribute to the model, such as a history of optic neuritis and race, are not included.

^cRefers to any subject who experienced more than 1 attack of transverse myelitis regardless of whether they were otherwise characterized as having opticospinal or typical multiple sclerosis.

*DRB1*1503* alleles, together or separately, did not contribute to the model, consistent with prior observations.³² Thus, another gene or genes of African origin within the HLA locus must contribute to disease severity.

Using survival analysis, we found similar results for the major MS disability milestone: the risk of requiring a cane to ambulate (EDSS score=6). African origin at HLA was associated with a 1.50-fold increased hazard ratio for cane dependency (**Table 5**). The hazard ratio was modified only slightly (hazard ratio, 1.36) by other statistically significant clinical variables (age at onset, brainstem/cerebellar presentation, transverse myelitis, and motor symptoms at onset), demonstrating that African origin at HLA contributes to disability progression independently of other recognized influences on MS disability. Use of interferon beta, glatiramer acetate, cytotoxic medications, and other therapies was initially included in the model as a time-dependent covariate. However, because the treatment histories between African American and white patients were well balanced, the time-dependent treatment

Table 5. Genetic and Phenotypic Influences on Disability as Measured by Survival Analysis Using Cox Proportional Hazard Models for Time to Cane Dependency^a

Characteristic	HR (95% CI)	P Value
Unadjusted analysis, African origin at HLA	1.50 (1.33-1.68)	<.001
Adjusted analysis		
African origin at HLA	1.36 (1.20-1.54)	<.001
Age at onset, per year	1.04 (1.03-1.05)	<.001
Brainstem/cerebellar presentation (n=169)	1.87 (1.25-2.80)	.002
Transverse myelitis (n=254)	1.54 (1.19-1.99)	.001
Motor symptoms at onset (n=342)	1.67 (1.29-2.15)	<.001

Abbreviations: CI, confidence interval; HR, hazard ratio.

^aTime to needing a cane to walk was determined by an Expanded Disability Status Scale score of 6. Subjects seropositive for anti-aquaporin 4 (n=11) were excluded, which did not substantially affect the HRs (data not shown).

covariates did not contribute to the model and were removed. Interestingly, genome-wide African ethnicity, which is associated with a 1.96-fold increase in the hazard ratio for cane dependency, did not retain independent significance, emphasizing the observation that much of the effect of African ethnicity on disability is accounted for by African origin at HLA.

CORRELATIONS WITH AGE AT ONSET

Subjects with *DRB1*15* had a 2.1-year younger mean age at disease onset compared with subjects with other *DRB1* alleles (**Table 6**). A gene-dosage effect of *DRB1*15* haplotypes on age at onset may be present. Linear regression modeling was used to investigate whether the difference in age at onset in African Americans is explained by the lower frequency of *DRB1*15* alleles in this population. Only a minor part of the difference in the age at onset between African American and white patients is accounted for by the difference in frequency of *DRB1*15* alleles in these populations. Subjects who developed optic neuritis at any time during their disease had a 3.3-year younger age at onset compared with those who did not develop optic neuritis

($P < .001$). Patients with a primary progressive disease course had an older age at onset compared with those with relapsing disease ($P < .001$).

COMMENT

We found carriers of DRB1*15 alleles were twice as likely to have classic/multifocal MS rather than the opticospinal subtype. Furthermore, the few individuals with opticospinal MS who were seropositive for anti-AQP4 antibodies never carried the DRB1*15 allele. We also report applying admixture mapping, a promising strategy for the identification of genetic susceptibility factors in complex traits,^{23,24,33} to the study of the phenotypic variation in the expression of MS. We show that African ancestry at HLA correlated with disability independently of DRB1*15 alleles. Lastly, DRB1*15 alleles were associated with an earlier age at onset.

The association of DRB1*15 with classic/multifocal disease course, but not the opticospinal subtype, suggests that from an immunogenetic perspective, these disorders are different. It remains to be determined whether other HLA alleles or different genes are associated with the opticospinal phenotype. Although this study did not have adequate statistical power to fully investigate the genetics of the anti-AQP4 seropositive phenotype, the observation that none of the 11 subjects who were seropositive for anti-AQP4 ever carried the DRB1*15 allele is intriguing and warrants further investigation. Studies of opticospinal MS in Japan, where the opticospinal subtype is relatively more common, suggest that there is an association with the centromeric *HLA-DPB1* gene,^{34,35} a possibility not addressed in this study. Additional work is needed to determine whether opticospinal MS and NMO will share HLA associations, which would suggest a continuous spectrum between opticospinal MS and NMO, or whether they will each have different HLA associations, implying immunogenetic distinctness. Given the important clinical implications of this genetic heterogeneity, multicenter collaborative efforts are warranted to amass a large enough data set to adequately address the genetic contributions to the opticospinal and NMO phenotypes.

To our knowledge, this is the first study in MS genetics that systematically examined its cohort for the opticospinal phenotype and AQP4 seropositivity. Although we attempted to distinguish patients with opticospinal MS and recurrent transverse myelitis from those with NMO by using published diagnostic criteria,³¹ approximately 5% of these patients tested seropositive for the anti-AQP4 antibody. It is possible that other MS cohorts used for genetic studies contain AQP4-seropositive subjects. Subsequent studies of MS genetics will need to address these phenotypes, especially if NMO is ultimately proven to be a separate disease rather than a subtype of MS.

Independently of DRB1*15 alleles, African ancestry at HLA correlated with disability as measured by MSSS and risk of cane dependency. These data indicate that a severity modifier gene or genes encoded at 6p21 exist. Genes within the HLA region that might contribute to severity include *HLA-DRB5*,³⁶ *HLA-C*,³⁷ and *TNF*.³⁸ The possible involvement of *DRB5* as a disease-severity modifier is sup-

Table 6. Genetic and Phenotypic Influences on the Age at Disease Onset^a

Analysis	Coefficient (95% CI)	P Value
Unadjusted analysis, DRB1*15 (n=641)	-2.09 (-3.07 to 1.11)	<.001
Adjusted analysis		
DRB1*15 (n=641)	-1.50 (-2.44 to -0.55)	.002
DRB1*15 heterozygous (n=548)	-1.38 (-2.36 to -0.40)	.006
DRB1*15 homozygous (n=93)	-2.19 (-4.10 to -0.28)	.03
African American race (n=665)	2.42 (1.48 to 3.36)	<.001
Optic neuritis (n=604)	-3.27 (-4.22 to -2.33)	<.001
Progressive onset (n=79)	7.33 (5.31 to 9.34)	<.001

Abbreviation: CI, confidence interval.

^aSubjects seropositive for anti-aquaporin 4 (n=11) were excluded, which did not substantially affect the hazard ratios (data not shown).

ported by work in transgenic mice that carry combinations of human DRB1*1501 and *DRB5*.³⁶ Dense SNP maps across HLA are becoming available and have the potential to identify the critical variants responsible for influencing disease severity in MS.

That DRB1*15 alleles reduce the age at onset is consistent with the proposed role of this allele as a susceptibility factor for MS and was previously reported in other cohorts.^{9,39,40} Alleles that exert a robust effect on MS susceptibility may be expected to lower the average age at onset by reducing time from exposure to an environmental trigger to onset of clinical symptoms. The older age at onset observed in African Americans³ may reflect an inherent resistance to developing MS.^{21,22} The DRB1*15 allele appears to counteract this inherent resistance, increasing the susceptibility for developing MS and lowering the age at onset in African Americans. Interestingly, the older age at onset of MS in African Americans is explained in part by the relatively lower frequency of DRB1*15 alleles in this population, underscoring how genetic factors influence not only the phenotypes of individuals but also populations.

Although not the subject of this study, environmental factors could contribute to the MS phenotypes. Environmental factors, such as exposure to the Epstein-Barr virus, sunlight, and vitamin D and smoking history, were implicated in MS susceptibility.⁴¹ Differences in these or other environmental factors between African American and white patients were not investigated in this study and could have important influences on the clinical outcomes we explored.

In summary, genetic variation at the major MS susceptibility gene, *DRB1*, as well as in other African origin genes around HLA, may account for a substantial fraction of the severity differences observed between African American and white individuals. These findings have 2 major therapeutic implications. First, the identification of a gene(s) within the major histocompatibility complex that contributes to MS severity may yield novel targets for therapeutic intervention. Second, demonstration that DRB1*15 influences clinical outcomes provides additional support for the potential value of therapies that regulate DRB1*15 molecules.⁴² Fine-mapping studies of HLA are under way and hold the promise of identifying an elusive MS severity gene.

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Author Affiliations: Department of Neurology, University of California–San Francisco, San Francisco (Drs Cree, Hauser, and Oksenberg, and Ms Tong); Department of Genetics, Harvard Medical School, Cambridge, Massachusetts (Dr Reich); Department of Neurology, Wayne State Medical School, Detroit, Michigan (Dr Khan); Harvard Medical School/Partners Healthcare Center for Genetics & Genomics, and Center for Neurologic Diseases, Department of Neurology, Brigham & Women's Hospital, Boston (Dr De Jager); Department of Neurology, Tohoku University School of Medicine, Sendai, Japan (Drs Nakashima and Takahashi); and Montreal Neurological Institute, McGill Medical School, Montreal, Quebec, Canada (Drs Nakashima and Bar-Or).

Correspondence: Bruce A. C. Cree, MD, PhD, MCR, Multiple Sclerosis Center, University of California–San Francisco, 350 Parnassus Ave, Ste 908, San Francisco, CA 94117 (bruce.cree@ucsf.edu).

Author Contributions: *Study concept and design:* Cree, Hauser, and Oksenberg. *Acquisition of data:* Cree, Khan, Takahashi, Bar-Or, Tong, and Hauser. *Analysis and interpretation of data:* Cree, Reich, De Jager, Nakashima, Hauser, and Oksenberg. *Drafting of the manuscript:* Cree, Khan, Tong, Hauser, and Oksenberg. *Critical revision of the manuscript for important intellectual content:* Cree, Reich, Khan, De Jager, Nakashima, Takahashi, Bar-Or, Hauser, and Oksenberg. *Statistical analysis:* Cree, Reich, and De Jager. *Obtained funding:* Cree, Hauser, and Oksenberg. *Administrative, technical, and material support:* Cree, Khan, Takahashi, Bar-Or, Tong, Hauser, and Oksenberg. *Study supervision:* Cree, Khan, Hauser, and Oksenberg.

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Announcement

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