HLA-DRB1*14 is a protective allele for multiple sclerosis in an admixed Colombian population

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ABSTRACT

Objective: The aim of this study was to determine ancestry informative markers, mitochondrial DNA haplogroups, and the association between HLA-DRB1 alleles and multiple sclerosis (MS) in a group of patients from Bogotá, Colombia.

Methods: In this case-control study, genomic DNA was isolated and purified from blood samples. HLA-DRB1 allele genotyping was done using PCR. Mitochondrial hypervariable region 1 was amplified and haplogroups were determined using HaploGrep software. Genomic ancestry was estimated by genotyping a panel of ancestry informative markers. To test the association of HLA polymorphisms and MS, we ran separate multivariate logistic regression models. Bonferroni correction was used to account for multiple regression tests.

Results: A total of 100 patients with MS (mean age 40.4 ± 12 years; 70% females) and 200 healthy controls (mean age 37.6 ± 11 years; 83.5% females) were included in the analysis. Ancestry proportions and haplogroup frequencies did not differ between patients and controls. HLA-DRB1*15 was present in 31% of cases and 13.5% of controls, whereas HLA-DRB1*14 was present in 5% of cases and 15.5% of controls. In the multivariate model, HLA-DRB1*15 was significantly associated with MS (odds ratio [OR] = 3.05, $p < 0.001$), whereas HLA-DRB1*14 was confirmed as a protective factor in our population (OR = 0.16, $p = 0.001$).

Conclusions: This study provides evidence indicating that HLA-DRB1*15 allele confers susceptibility to MS and HLA-DRB1*14 allele exerts resistance to MS in a highly admixed population. This latter finding could partially explain the low prevalence of MS in Bogotá, Colombia. *Neurol Neuroimmunol Neuroinflamm* 2016;3:e192; doi: 10.1212/NXI.0000000000000192

GLOSSARY

AIM = ancestry informative marker; **HVR1** = hypervariable region 1; **HU-FSFB** = Hospital Universitario Fundación Santa Fe de Bogotá; **MHC** = major histocompatibility complex; **MS** = multiple sclerosis; **mtDNA** = mitochondrial DNA; **OR** = odds ratio.

The most consistent genetic risk factor reported in multiple sclerosis (MS) is the HLA-DRB1*15 allele, which has been found in different populations, including Europeans,[1,2] African Americans,[3] and Latin Americans.[4–6] A protective genetic effect in European and non-European populations has also been reported with other class II major histocompatibility complex (MHC) alleles, including HLA-DRB1*01, HLA-DRB1*10, HLA-DRB1*11, and HLA-DRB1*14.[7,8]

Ancestry can be estimated using nuclear ancestry informative markers (AIMs), which are insertion/deletion polymorphisms with high genomic allele frequency differences between populations.[9] Furthermore, mitochondrial DNA (mtDNA) haplogroups are markers used to assess matrilineal history.[10] Ancestry can be inferred more accurately using both AIMs and mtDNA haplogroups and can be used for population stratification, which is particularly important in recently admixed populations such as African Americans and Latin Americans.[11]

The city of Bogotá, Colombia is located in the subtropical region near the equator. The prevalence of MS in Bogotá is 4.4 per 100,000, making it a low-risk area.[12] In contrast, MS prevalence has been reported to be higher in southern regions such as New Zealand[13] and in northern regions such as Northern Europe,[14] where it can exceed 200 per 100,000 inhabitants.[15]

Genetic association studies of MS in Colombia and other Latin American countries are scarce. Considering the low prevalence of MS in Colombia, we hypothesized we would find a protective allele in our population. This case-control study assessed whether HLA-DRB1 alleles were associated with susceptibility or resistance to MS in patients from the low MS prevalence and highly admixed population of Bogotá.

METHODS **Standard protocol approvals, registrations, and patient consents.** The study protocol was approved by the institutional review boards of Hospital Universitario Fundación Santa Fe de Bogotá (HU-FSFB), Universidad de los Andes, and Universidad El Bosque. All participants provided written informed consent before all the procedures.

Study population. Patients diagnosed with clinically definite MS according to the 2005 McDonald criteria[16] were recruited from 2007 to 2013 at the neurology clinic of the HU-FSFB, which is a tertiary referral center that provides medical care to patients with MS from all over Colombia. A neurologist (J.T.) with expertise in MS confirmed the diagnosis in all patients. Healthy controls without past medical history of autoimmune disease or other neurologic conditions were recruited by community advertisement within 1 week of enrollment of a given case. To exclude any MS-related signs or symptoms, controls underwent neurologic examination by one of the authors. Patients included were residents of Bogotá, Colombia and were older than 18 years at the time of enrollment. The following variables were recorded for each patient: sex, age at disease onset for cases and age at enrollment for cases and controls, smoking history (ever vs never), family history of MS, disease duration, and clinical phenotypes.

DNA extraction. Venous blood was collected and DNA was extracted using the FlexiGene DNA kit (QIAGEN, Hombrechtikon, Switzerland). All samples were stored at −20°C until their use, and DNA concentration was determined by spectrophotometry using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA). Genotyping was done at the Human Genetics Laboratory of Universidad de los Andes.

Molecular assessment of AIMs. A panel of 46 AIMs was amplified in a single multiplex PCR followed by capillary electrophoresis, as previously described.[9] Dye-labeled amplified fragments were separated and detected using an ABI Genetic Analyzer 3500 (Life Technologies, Carlsbad, CA), and allele calls were obtained with GeneMapper v4.1 (Life Technologies). Ancestral proportions were calculated using the STRUCTURE software v2.3.2. Considering the historical formation of Colombia, we assumed a trihybrid contribution from Native

Americans, Europeans, and Africans.[17] The Human Genome Diversity Cell Line Panel was used for reference.[9]

mtDNA haplogroups. The mtDNA hypervariable region 1 (HVR1) was amplified by PCR with the L15840 (5′ACTTCACAACAAATCCTAATCCT3′) and H16436 (5′CGGAGCGAGGAGAGTAGCAC 3′) primers. Amplicons were sequenced using an ABI-PRISM Dye Terminator Cycle sequencing system (Applied Biosystems, Foster City, CA). Sequence variants were determined between mtDNA nucleotides HVR1 (16024–16365) by comparing them with the revised Cambridge Reference Sequence (GenBank accession no. NC_012920.1) using HaploSearch software.[18] The haplogroup classification was inferred using HaploGrep software.[19]

HLA typing. The samples were genotyped at the HLA-DRB1 gene locus using the HLA-DRB sequence-specific primer PCR kit (Biotest AG, Dreieich, Germany). Each reaction included primers for a fragment from the human growth hormone gene as internal controls. In the final data set, the alleles were resolved into 13 allele groups. For more details, see the e-methods at Neurology.org/nn.

Statistical analysis. Descriptive statistics, including mean, median, SD, and interquartile range, were used to characterize the study population. Group comparisons of demographic and clinical characteristics were performed using Z-test or Wilcoxon rank sum test where appropriate. When comparing proportions of categorical variables, a Z-proportion test, χ^2 test, and Fisher exact test were used as appropriate. A p value <0.05 was considered statistically significant.

To assess population stratification, a k-means cluster analysis of ancestry data was performed. Odds ratios (ORs) were used to reflect effect sizes of MS risk. A stepwise selection logistic regression analysis was performed starting with a full model, which had a significant level of addition and removal of 0.1 and 0.25, respectively. To control for type I error after 13 different tests, the Bonferroni correction was applied, and a p value <0.004 was considered statistically significant. The Hosmer-Lemeshow statistic and deviance were used to measure the goodness of fit of the model to the data set. Hardy–Weinberg equilibrium was calculated using asymptotic tests[20] (STATA v12.0 software; StataCorp, College Station, TX). All analyses were performed using Stata 11 SE.

RESULTS A total of 103 patients and 202 controls were enrolled. However, we included in the analysis only the 100 patients and 200 controls in whom mtDNA, AIMs, and HLA genotyping was successful. Demographic data of the study population are shown in table 1.

mtDNA haplogroups and MS. A total of 12 mtDNA haplogroups were identified in the patients studied. The Amerindian haplogroup A was the most common in our population; it was present in 36% of the patients and 45% of the healthy controls. The Amerindian haplogroup B was present in 24% of the patients and 26.5% of the healthy controls. Amerindian haplogroups C and D were each present in 12% in the patients; haplogroup C was present in 9.5% of controls and haplogroup D was present in 7.5% of controls. The African haplogroup L was found in 5% of the patients and 6.5% of the healthy controls. The European haplogroups H, J, T, U, V,

Table 1 Clinical and demographic features of the enrolled participants

	MS (n = 100)	Controls (n = 200)	p Value
Sex, female, n (%)	70 (70)	167 (83.5)	0.0068[a]
Age, y, median (IQR)	39 (30.5-48.5)	38 (29-46)	0.0836[b]
Age at onset, y, median (IQR)	30 (23-29)	–	
Age at disease diagnosis, y, median (IQR)	32 (25-41)	–	
Smoking history, n (%)	39 (39)	61 (30.5)	0.1410[a]
Family history of MS, n (%)	3 (3)	3 (1.5)	0.3817[a]
Clinical phenotypes, n (%)			
Relapsing-remitting	81 (81)	–	
Secondary progressive	14 (14)	–	
Primary progressive	4 (4)	–	
Progressive-relapsing	1 (1)	–	

Abbreviations: IQR = interquartile range; MS = multiple sclerosis.
Smoking history: ever vs never. Family history is defined as presence of primary or secondary relatives with MS.
[a] χ² test.
[b] Wilcoxon rank sum test.

and X and the Asian haplogroup Y were less commonly found in our population and accounted for less than 5%. However, the distribution of mtDNA haplogroups among patients and healthy controls was not different ($p = 0.218$).

AIMs and MS. Ancestry estimation revealed that patients with MS had on average 55.03% European, 32.55% Amerindian, and 12.42% African ancestry, whereas healthy controls had 54.71% European, 34.28% Amerindian, and 11.01% African ancestry. Cluster analysis revealed no differences among patients and controls when comparing the European ($p = 0.872$), Amerindian ($p = 0.992$), or African ($p = 0.204$) ancestry contribution.

Association analysis for HLA alleles. No deviation from Hardy–Weinberg equilibrium was seen in the data set ($p > 0.01$). Results of the stepwise selection modeling identified the following variables as covariates in the multivariate logistic regression model: age at onset of symptoms (age at enrollment for the healthy controls), sex, smoking history, and ancestry (European and Amerindian proportion). After controlling for admixture, the association between the multiallelic HLA-DRB1 and MS was confirmed. HLA-DRB1*04 allele was the most frequent in both patients (42%) and controls (42.5%), whereas HLA-DRB1*09 and HLA-DRB1*12 alleles were the least frequent (see table 2). HLA-DRB1*15 was present in 31% of cases and 13.5% of controls, whereas HLA-DRB1*14 was present in 5% of cases and 15.5% of controls. The presence of HLA-DRB1*15 was associated with MS onset in the univariate model (OR = 2.87, $p < 0.001$) and multivariate models (OR = 3.05, $p < 0.001$). HLA-DRB1*14 was not associated with MS in the univariate analysis (OR = 0.28, $p = 0.012$) but reached statistical significance in the multivariate model and was confirmed as a protective factor (OR = 0.16, $p = 0.001$) in our population. No significant association was found for other HLA-DRB1 alleles.

Table 2 Association of HLA-DRB1 alleles and MS

HLA-DRB1 allele	MS, n (%) (n = 100)	Controls, n (%) (n = 200)	Univariate analysis			Multivariate analysis		
			OR	95% CI	p Value	OR[a]	95% CI	p Value
*01	14 (14)	25 (12.5)	1.13	0.56-2.30	0.716	1.25	0.59-2.67	0.549
*03	21 (21)	36 (18)	1.21	0.66-2.20	0.533	1.25	0.65-2.38	0.492
*04	42 (42)	85 (42.5)	0.97	0.60-1.59	0.934	1.00	0.59-1.70	0.972
*07	17 (17)	37 (18.5)	0.90	0.47-1.69	0.750	0.99	0.50-1.96	0.983
*08	9 (9)	26 (13)	0.66	0.29-1.47	0.312	0.61	0.25-1.45	0.271
*09	1 (1)	4 (2)	0.49	0.05-4.48	0.532	0.27	0.02-2.70	0.269
*10	3 (3)	5 (2.5)	1.20	0.28-5.15	0.800	1.87	0.40-8.60	0.419
*11	8 (8)	29 (14.5)	0.51	0.22-1.16	0.112	0.51	0.21-1.23	0.138
*12	1 (1)	4 (2)	0.49	0.05-4.48	0.532	0.27	0.02-2.78	0.275
*13	24 (24)	43 (21.5)	1.15	0.65-2.03	0.624	1.11	0.60-2.06	0.719
*14	5 (5)	31 (15.5)	0.28	0.10-0.76	0.012	0.16	0.05-0.46	0.001
*15	31 (31)	27 (13.5)	2.87	1.60-5.17	<0.00	3.05	1.62-5.71	<0.001
*16	9 (9)	17 (8.5)	1.06	0.45-2.48	0.885	1.44	0.58-3.56	0.425

Abbreviations: CI = confidence interval; MS = multiple sclerosis; OR = odds ratio.
[a] Adjusted OR after controlling for the following covariates: age at onset of symptoms (age at enrollment for the healthy controls), sex, smoking history, and ancestry (European and Amerindian proportion).

DISCUSSION In this study exploring the association between HLA alleles and susceptibility to MS in Colombia, we also describe additional genetic data such as AIMs and mtDNA haplogroups. Our results were consistent with previously reported HLA-DRB1 allelic associations. Although HLA-DRB1*15 allele has been classically reported as a risk factor for MS in European descendants,[1] it has also been found in other Caucasian,[5] African,[3] and Latin American populations, including studies from Brazil,[5] Argentina,[21] and Martinique.[22] This allele has been shown to increase not only disease susceptibility[23] but also morbidity.[24] In the univariate analysis, we found a significant association between HLA-DRB1*15 and MS risk. Furthermore, this finding was sustained in the multivariate analysis and survived Bonferroni correction, conferring a 3-fold increased risk of MS among our population. Previous studies have found a similar effect size for HLA-DRB1*15.[25]

HLA-DRB1*14 allele exerts a protective effect in our population. The role of HLA-DRB1*14 observed in this study has been reported in other countries, such as Canada[7] and China.[7] Some studies have shown that its protective effect is strong enough to counteract the risk conferred by the presence of HLA-DRB1*15 and to influence the regional prevalence of MS.[7]

A previous study in the Antioquia region of Colombia also reported a significant association between HLA-DRB1*15 allele and MS. This study also identified HLA-DRB1*01 allele as a risk factor and HLA-DRB1*07 allele as a protective one.[26] However, we did not find these associations in our population. This difference could be partially attributed to our larger sample size and the statistical methods used in our study (e.g., adjusting for multiple confounders related to demographic and genetic characteristics).

The clinical and demographic characteristics of our patients were fairly consistent with those previously reported in other countries.[27] The Colombian population is thought to be genetically substructured, with different proportions of European, Amerindian, and African influxes depending on the region. AIM analysis showed that the population of Bogotá has a significant genetic admixture with predominant European and Amerindian ancestry. African ancestry was less frequent. Similar genetic composition has been found in other Colombian populations and can be explained by Colombia's colonization and slave trade history.[28] In addition, our population has the same ethnic origin as that of other Colombian groups, as the mtDNA analysis did not differ from previous studies.[29]

AIMs are mainly used to estimate ancestry proportions, allowing for assessment of the structure in admixed populations, and mtDNA haplogroups reflect a clear pattern of matrilineal historical events that are not obscured by the factors of recombination. Therefore, it has been suggested that using both genetic markers can improve the accuracy of the reconstitution of genetic ancestry among certain populations, particularly those that are highly admixed, like most in Latin America.[9]

AIMs can be used to perform genetic matching or to correct substructure effects in case-control studies.[9] Because there were no differences in AIMs and mtDNA haplogroups between patients and controls, we can assume that our study population is not stratified and that the observed associations with HLA-DRB1 alleles are not spurious.

Because previous studies conducted in Latin America[26,30] have consistently reported significant associations between the *HLA-DRB1* gene and MS risk, we did not include other protective and predisposing alleles such as HLA-A*02:01 and HLA-DQB1*06:02, respectively, which would have allowed us to more comprehensively evaluate their genetic influence on MS susceptibility in Colombia. Furthermore, it would have been clinically relevant to explore the complex interaction between genetic expression, disease severity, and environmental factors; however, this was outside the scope of this study and is the subject of ongoing investigation in our group.

The increased number of HLA alleles reported across different ancestral groups has led to the conclusion that immunologically relevant genes across the MHC region dominate the genetic contribution of MS. However, additional efforts must be made to validate current and future findings in genetic-matched populations using AIMs and mtDNA haplogroups. This study provides evidence indicating that HLA-DRB1*15 allele confers susceptibility to MS whereas HLA-DRB1*14 allele exerts resistance to MS in the low prevalence and highly admixed population of Bogotá, Colombia.

AUTHOR CONTRIBUTIONS

interpretation of data. Diana M. Narváez: drafting/revising the manuscript for content, including medical writing for content; study concept; and acquisition, analysis, and interpretation of data. Wilmer Cárdenas: design and conceptualization of the study. Alexandra Porras: analysis and interpretation of the data. María-Claudia Lattig: design and conceptualization of the study. Helena Groot de Restrepo: drafting/revising the manuscript for content, including medical writing for content, and design and conceptualization of the study.

DISCLOSURE

J. Toro is associate editor of *NEJM Journal Watch Neurology* and is an editorial board member for *Multiple Sclerosis and Related Disorders*. D. Cuellar-Giraldo, C. Díaz-Cruz, L.-E. Burbani, C.-M. Guío, S. Reyes, F. Cortes, S. Cárdenas-Robledo, D.M. Narváez, W. Cárdenas, A. Porras, M.-C. Lattig, and H. Groot de Restrepo report no disclosures. Go to Neurology.org/nn for full disclosure forms.

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This information is current as of December 24, 2015

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