INVESTIGACIÓN / RESEARCH

HLA-DRB1*1101 allele confer protection for Multiple Sclerosis disease in Cuban population

Alelo HLA –DRB1*11:01 asociado con la Esclerosis Múltiple en población cubana confiere protección

Fernández-de-Cossío ME, [1*]Cintado A, [1] Nazabal M,[1] Hanlet C,[1] Díaz T,[1] Villarreal A,[1] Ale M,[1] Grass D, [2] Cervantes-Llanos M,[1] Pavon-Fuentes N, [2] Benitez JV, [1] Cabrera-Gomez JA,[2] Diaz de la Fe A,[2] Pentón-Rol G. [1]

*Maria Elena Fernández-de- Cossio and Alberto Cintado-Benitez contributed equally to this work.

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ABSTRACT

Multiple sclerosis is a neuro-inflammatory autoimmune disease. The MHC class II region produces the strongest effect on Multiple Sclerosis genetic susceptibility. The purpose of this study is to evaluate DRB1*, DQA1*, and DQB1* alleles among Multiple Sclerosis Cuban patients. 100 patients and 200 unrelated healthy controls were included. Ancestry informative markers were used. Multiple Sclerosis associated HLA susceptibility/protection alleles were ascertained by PCR using specific primers. Statistical analyses were conducted using STRUCTURE 2.1, ADMIXMAP 3.7, SPSS 16.0 packages. Logistic regression analysis showed odds ratio of 5.7 for Multiple Sclerosis associated with a unit change in European admixture proportion. Evidence for susceptibility to Multiple Sclerosis was observed for the presence of HLA-DRB1*15, DRB1*14, DQA1*01 and DQB1*06 alleles, with odds ratio 3.40, 4.96, 2.53, and 2.77 respectively compared to healthy controls. A protective effect of HLA DRB1*01, DRB1*07, DRB1*10 was found among Multiple Sclerosis patients (odds ratio 0.35, 0.2, 0.4 respectively). After correcting for admixture, a new association to HLA-DRB1*1101 was identified. Alleles HLA-DRB1*, DQ were associated with Multiple Sclerosis in Cuban patients. HLA-DRB1*1101-1104 was associated to Multiple sclerosis in this study sample, where the European ancestry proportion was identified.

Keywords: HLA-DR, HLA-DQ, Admixture, Multiple Sclerosis, Cuban population, AIM

RESUMEN

La Esclerosis Múltiple (EM) es una enfermedad autoinmune neuroinflamatoria. La región clase II del Complejo Mayor de Histocompatibilidad (CMH) produce el mayor efecto genético sobre la EM. El objetivo de este estudio es evaluar los alelos DRB1*, DQA1* y DQB1* en enfermos cubanos con EM. En el presente estudio se incluyeron 100 pacientes y 200 individuos no relacionados. Se emplearon Marcadores informativos de ascendencia. Se realizó la determinación de los alelos HLA, indicadores de protección-susceptibilidad, asociados con la EM mediante la reacción en cadena de la polimerasa y el uso de oligonucleóticos específicos. Los análisis estadísticos se desarrollaron con los paquetes estadísticos STRUCTURE 2.1, ADMIXMAP 3.7 y SPSS 16.0. El análisis de regresión logística mostró un valor de odds ratio de 5,7 para una asociación de la EM con la proporción de mezcla europea en una unidad de cambio. Los alelos HLA-DRB1*15, DRB1*14, DQA1*01 y DQB1*06, confieren susceptibilidad a la EM con valores de *odds ratio* de 3.40, 4.96, 2.53, y 2.77 respectivamente. Un efecto de protección se observó para los alelos HLA DRB1*01, DRB1*07, DRB1*10 con *odds ratio* de 0.35, 0.2, 0.4 respectivamente. El alelo DRB1 * 1101-1104 se asoció a la EM en la muestra de estudio, donde se identificó la proporción de ascendencia europea.

Palabras clave: HLA-DR, HLA-DQ, mezcla genética, Esclerosis Múltiple, población cubana, marcadores informativos de ascendencia

Introducción

Multiple sclerosis (MS) is a chronic neuro-inflammatory autoimmune disease believed to arise from complex interactions of both environmental and genetic factors. A genetic contribution to the pathogenesis of MS had already been assumed in 1896 with the discovery of the familial aggregation of MS.[1] The earliest association between genes and MS found in the Human leukocyte antigen[2] (HLA) and described in 1972 at 6p21 has long been recognized as the strongest locus increasing risk to MS in most populations,[3] nota-

[1] Pharmacogenomics Department. Biomedical Research Division. Center for Genetic Engineering and Biotechnology, 31 entre 158 & 190, Cubanacán, Playa, Cuba.
[2] International Center of Neurological Restoration. Ave 25 no 15805 entre 158 & 160 Cubanacan Playa, Cuba.

Correspondence: email: mariaelena.fernandez@cigb.edu.cu

bly HLADRB1*1501 and other major histocompatibility complex (MHC) alleles.[4]

An association was seen for the DRB1*1501 allele, as the main susceptibility allele in MS residing on a large, extended haplotype.[5] This has been fine-mapped to the HLA-DRB5*0101-DRB1*1501-DQA1*0102-DQB1*0602 extended haplotype in the Northern European population with estimated risk ratios of approximately 3, and homozygozity for this haplotype increases MS risk sixfold.[6]

The overall prevalence of MS is about 70 per 100,000 individuals (range from 2 to 150).[7] The first case of MS in Cuba was published in 1965. After 1990, the prevalence of clinically–definite MS in the different Cuban provinces was around 10 per 100,000. The latest study, in Cienfuegos province,[8] provides a prevalence of 25.5 per 100,000.

MS has been reported in all continents in different populations. Of these, Europe is considered a high prevalence region for MS (≥ 30/100000) and different attempts are undergoing to evaluate how the risk of MS varies among European populations. [9] Brum et al., have defined that MS presents a high prevalence rate, particularly in white persons from Western countries when patients from Brazil were analyzed.[10]

Cintado et al., have stated a population structure in a multiethnic Cuban population from Havana City and they have reported that 85% are of Spanish descent and the African contribution is of 15% suggesting that the admixture must be considered when evaluating traits in this population.[11] They have outlined that differing frequencies of certain diseases in European populations compared with African and Amerindian populations suggest that those of Spanish descent may be particularly useful for deciphering complex genetic diseases, for instance dementia and multiple sclerosis that have a higher risk in European than in African populations.[12]

The purpose of this study is to assess, for the first time, DRB1*, DQA1*, and DQB1* alleles among MS Cuban patients to understand the nature of the HLA association with the diseases in our sample. Also, we typed a panel of 17 Ancestral informative markers (AIM) to control possible confounding traits by population stratification.

Material and methods

Patients and controls

Samples from 100 MS patients with Relapsing Remitting (MS-RR) diagnosed according to McDonald et al., were analyzed in the present study.[13] Unrelated healthy control (n= 200) were matched in terms of age, gender and ethnicity with the group of patients enrolled in the study. Written informed consent of each subject was obtained prior to sample collection. The study protocol was approved by the Ethical Research Board from the International Center of Neurological Restoration (Havana, Cuba) according to the guidelines of the national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association.

Subject characteristics are shown in Table 1. The mean age of MS patients at onset was 40.01+/-13.09 years; 71.04% were woman. This study was approved by the Ethical Research Board from the International Center of Neurological Restoration (Havana, Cuba).

DNA isolation and AIM genotyping

Genomic DNA was isolated from whole blood using a Wizard Genomic DNA Purification Kit according to the manufacturer's instruction (Promega, Madison, WI, USA). Table 2 shows the 17 AIM analyzed in this study. AIM were identified from previous studies as having large differences in allele frequency (d. 30%) between Native American, West African, and European ancestral populations.[14] Genotyping was performed by PCR amplification, using sequence-specific primers (PCR-SSP), as previously described.[15] Amplification reactions were performed in a final volume of 25 µL containing 100 ng of genomic DNA, 1.5–2.0 mmol/L MgCl2, 50 mmol/L KCl, 10 mmol/L Tris HCl, 0.2 mmol/L of each dNTP, 1mmol/L of both primers, 10% DMSO, and 1UTaqDNApolymerase (Promega). All the loci were scored after electrophoresis through agarose gels.

Table 1. Demographic characteristics of study subjects

Characteristic	Cases	Controls
No. of subjects (n)	100	200
Gender (female %)	71.04	79,12
Age (mean +/- SD)	40.01+/- 13.09	38.6 +/- 12
*Self-identified ethnicity (%)		
White descent	76	72.5
Black descent	10,6	12,5
Mulattos	13,3	15

* *Self-identified ethnicity*[12]

HLA Typing.

Selected HLA DRB1 and DQ alleles were determined by SSP-PCR as previously described.[16, 17] The selection of these particular alleles was based on previous studies in which they have been reported to be implicated as part of haplotypes conferring the highest risk or protection scores5. [18] Brief description: DRB1* 0401-0411-0301-1501-1502 -0101-0103 -0102 -0701-0702 -1001 -1101-1104 -1401-1404 1403, DQB1* 0602, DQA1* 0301, *0102. A primer pair to amplify the third intron of DRB1 genes was included in each PCR reaction as the internal positive control. These two primers matched non-allelic sequence. [17, 18] All primers were obtained from the Department of oligonucleotide synthesis, at Center of Genetic Engineering and Biotechnology (Havana, Cuba), and used at 0.25 µM. Amplified products were separated by electrophoresis in 2 % agarose gels containing ethidium bromide after the addition of loading buffer, and visualized using UV illumination.

Table 2. AIM allelic frequency in the parental populations[15]. AIM allelic frequencies for MS subjects and controls are also shown. Asterisks precede the name of the larger allele.

AIMs	European	West Africans	Amerindian	Cases	Controls
GC-*1F	0.356	0.853	0.339	0.520	0.586
AT3*ins	0.273	0.858	0.061	0,450	0,430
LPL*ins	0.494	0.971	0.442	0.480	0,630
APOA1*ins	0.917	0.420	0.977	0.800	0,768
MID154*ins	0.333	0.806	0.420	0,378	0,415
MID187*ins	0.342	0.759	0.301	0.430	0.442
D11S429*T	0.440	0.087	0.119	0.430	0.375
TSC11020*T	0.921	0.487	0.137	0.830	0,824
FY-null*T	0.999	0.001	1.000	0,800	0,715
OCA2*A	0.636	0.115	0.488	0.582	0.563
WI-7423*T	0.517	0.000	0.058	0.516	0.340
GS*1S	0.607	0.931	0.931	0.760	0.758
WI-14867*C	0.558	0.976	0.418	0.620	0.571
WI-16857*G	0.180	0.751	0.181	0.256	0.390
PV92*ins	0.171	0.225	0.792	0,25	0,196
CYP19-E2*T	0.287	0.332	0.741	0,239	0,419
TYR 192*A	0.485	0.005	0.034	0.400	0.359

Statistical analysis. The fit of the genotype frequencies to the Hardy-Weinberg proportions was tested by the Chi-square test. Haplotype frequencies were estimated by means of an expectation maximization algorithm.[19] We used parental population frequencies reported[14] for samples of Spanish, average of Amerindians (Mayan, southwestern US Native Americans), and average of West Africans (Central African Republic, Nigeria, and Sierra Leone) (Table 2). To test for population stratification, the STRUCTURE 2.1 program[19] was used. The program was run initially with 70000 iterations for the burn-in period and 100000 additional iterations to obtain parameter estimates, with a prior distribution that allowed K to take values from 1 to 3. Additional runs with longer iterations were also carried out to check the consistency of the results. The frequencies of HLA class II was compared between patients and controls using the chi-square test or the two-tailed Fisher's exact probability test. Odds Ratio (OR) (95 % CI) was also estimated (SPSS 16.0 software). The level of significance was taken as p value < 0.05. For allele comparisons, Bonferroni's method was used for the correction of multiple comparisons, multiplying the value of p obtained in the statistical test by the total number of alleles tested.[16] In order to control potential confounding because of population stratification as a result of admixture, the ADMIXMAP program was used.[21] This program performs an association analysis by means of a logistic regression for the relation of MS, as the dependent variable, and HLA, introducing individual ancestry estimates as covariates. The admixture proportions of groups were estimated by the gene identity method[22] using the software Admix95 kindly provided by Bertoni B (http://www.genetica.fmed.edu.uy/software.htm)

Results

Allele frequencies for the 17 AIMs in ancestral populations, MS, and healthy controls are shown in Table 2. There was no deviation from Hardy-Weinberg proportions (p>0.05). Genetic ancestry estimates showed that European contribution was preponderant in patients and controls groups, 80.1% and 76% respectively. West African estimates reached 16.9% and Native American 2% for cases, while West African reached 21.7% and Native American 2.3% for controls. The majority of the cohort concentrates to the European component of the European-West African axis with a restricted and almost absent Native American component.

Our results indicate the presence of substructure in the whole sample. The STRUCTURE program consistently assigned individuals to two groups with a higher probability than it did to a single group or three groups. The probability of K=2 (-4257.7) was higher than K=1 (-4350) and K=3 (-4365), indicating the presence of two distinct clusters

The result of the logistic regression analysis with MS as a dependent variable showed that *odds ratio* for MS, associated with an unit change in European admixture proportion, was estimated as 5.7 (95% CI 1.2–36).

The association of selected HLA DR/DQ alleles with MS in the Cuban sample was evaluated. HLA-DRB1 and DQ typing differed significantly between MS patients and controls. The HLA-DRB1, HLA-DQA1 and DQB1 allelic associations for MS patients and controls are shown (Table 3). Evidence for susceptibility to MS was observed for the presence of HLA-DRB1*15, DRB1*14, DQA1*01 and DQB1*06 alleles, with higher odds ratio (OR) compared to healthy controls (Table 3). A modest protective effect of HLA DRB1*01, *07, *10 were found among MS patients (OR 0.35, 0.2, 0.4 respectively).

After correcting for admixture, a new association to HLA-DRB1*1101 was identified, and associations for the alleles above

Table 3. HLA Allelic association of MS patient's vs controls

HLA	Alleles	*p value* non adjustment for admixture	*p value* adjustment for admixture	*OR*	95% CI
DRB1*	*0301*	ns	ns	*0.86*	*(0.8-1.5)*
	1501-1502	<0.01	<0.01	*3.40*	*(1.8-6.1)*
	0101-0103	ns	ns	*0.35*	*(0.16-0.92)*
	0102	ns	ns	*0.24*	*(0.06-0.8)*
	0701-0702	<0.01	<0.01	*0.21*	*(0.1-0.5)*
	1001	ns	ns	*0.47*	*(0.25-0.91)*
	1101-1104	ns	<0.01	*0.19*	*(0.084-0.441)*
	1401-1404	<0.01	<0.01	*4.96*	*(2.29-10.7)*
	1403	ns	ns	*0.86*	*(0.25-2.92)*
DQA1*	*0301*	ns	ns	*1.25*	*(0.71-2.20)*
	0102	<0.01	0.01	*2.53*	*(1.48-4.32)*
DQB1*	*0602*	<0.01	<0.01	*2.77*	*(1.57-4.86)*

OR, odds ratio; CI, confidence interval; p value < 0.05 corrected for Bonferroni was considered significant; ns= not significant

Table 4. Association analysis of HLA haplotypes with the MS *p value* adjustment for admixture

Haplotype	Alleles	*p value*	*OR*
DQA1*/ DQB1*	*0102/ 0602*	0.003	2.2
DRB1*	*0103/ 0701/ 1001*	0.04	0.6
DRB1*/ DQA1*/ DQB1*	*1501/ 0102/ 0602*	$1,3x10^{-5}$	3.8
DRB1*/ DQA1*/ DQB1*	*1501-0103-0701-1001/ 0102/ 0602*	$0,6x10^{-3}$	3,22

p value < 0.05 corrected for Bonferroni was considered significant

described before admixture correction (p<0.01) remained significant.

The disease association at the level of four-loci haplotypes was carried out (Table 4). DQA1*0102/DQB1*0602 (p<0.003); DRB1* 1501/ DQA1* 0102/ DQB1*0602 and DRB1* 1501-0103-0701-1001/DQA1*0102/DQB1*0602 ($0.6x10^{-3}$) have odds ratios 2.2, 3.8 and 3.22 respectively, whereas DRB1* 0103/*0701/*1001 (p<0.04) has OR 0.6. These associations remained significant after correcting for admixture.

Discussion

MS is a complex autoimmune disease with over 100 confirmed associated genes or genetic loci and a low increasing number of confirmed environmental risk factors.[5] The strongest genetic predisposition correlates with the major histocompatibility complex, class II, DR beta 1 (HLA-DRB1*1501 allele) with some contribution from other alleles. [5]

We report that HLA-DRB1 and DQ typing significantly differed between MS patients and controls. Evidence for susceptibility to MS was observed with the presence of HLA-DRB1*15, DRB1*14, DQA1*01 and DQB1*06 alleles with higher odds ratio (OR) compared to healthy controls (Table 3). Diaz-Horta et al reported before an opposite association for diabetic Cuban patients. [23] Modest protective effects of HLA DRB1*01, DRB1*07, DRB1*10 were found among MS patients (OR 0.37, 0.2, 0.4 respectively).

Several genetic studies have described HLA-DRB1*1501 as the main susceptibility allele in MS6. Dyment et al., later considered the importance of taking into account that some haplotypes or loci other than HLA-DRB1*15 contribute to MS risk24. However, a recent genome wide association study5 that includes 9,772 MS patients and 1700 controls showed that DRB1*1501 has the strongest association with MS with a *p value* of 1x10-320. Our results agree with this report.

Other studies examined the role of ethnicity or ancestry in MS epidemiology[7, 25] A relatively high prevalence of MS is found in Western Europe and North America. In Europe the prevalence ranges from 10 to 187 per 100000 with a higher rate in northern countries.[26] In addition, a strong association between the HLA-DRB1*15 allele and MS has been shown in studies of northern Europeans and their descendants.[27]

The Cuban population is essentially a result of the mixture between Spanish, West Africans, and to a lesser degree, Amerindian tribes that inhabited the island.[28] Cintado et al., defined a population structure in a multiethnic Cuban population from Havana City and reported 85% of Spanish European descent.[11]

A typing of 17 markers having large differences in frequencies between European, West African and Native American populations was applied to the present study to associate ancestry proportions with respect to MS. We observed that European contributions predominate in patients that are mainly white (76%), with an estimated OR (*odds ratio*) of 5.7, with a wide-range confidence interval (CI 1.2-36).

Similar estimates were reported for MS patients in Brazil, also a country with a highly mixed population. According to the Brazilian study MS had a high prevalence within whites with Western Europe ancestry. [10] In Cuba, in respect to another autoimmune disease, it was confirmed that T1DM was associated with a preponderant genome proportion of European ancestry after ADMIXMAP evaluation.[23]

Toro et al have been studied the low prevalence of MS in Bogota, Colombia which present highly admixed population and have reported that HLADRB1*15 allele confers susceptibility to MS whereas HLA-DRB1*14 allele exerted resistance to disease. [29]

Results from the present study have defined another allele, HLA-DRB1*1101, associated to the disease, mainly in Caucasian patients. These outcomes confirm the importance of ancestry evaluation as a robust tool to adjust for population admixture, which controls population stratification and greatly avoids false associations in case-control studies.[30] The distribution and diversity of DRB1*11 alleles varies among populations. DRB1*1101 has been described as the predominant allele, while HLA DRB1*1104 is the second most frequent allele, as stated by a previous study that included Caucasian, Asian/Pacific Islanders, Hispanics and Native Americans. [31]

Even more, HLA-DRB1*1101 is part of certain allele groups defined for their association to MS risk , [7, 32] which is formed by the alleles DRB1*03, DRB1*14, DRB1*07 and DRB1*11. However in the present study this allele was associated to disease protection, a result that corresponds to reports from others authors [33] which have found that HLA-DRB1*14 and HLA-DRB1*11 bearing haplotypes are protective with OR 0.75 and 0.55 respectively. In that sense Ramagopalan et al., using a Canadian dataset confirmed that HLA DRB1*11 bearing haplotypes are novel and statistically significant resistance haplotypes. [34] They also defined that this allele shows a multiplicative mode of inheritance. According to these authors the protective effect of HLA DRB1*11 over HLA-DRB1*15 appears to be weaker than the effect of HLA –DRB1*14, but both alleles reduce risk associated with HLA-DRB1*17. Dominant variants on HLA-DRB1*14 and HLA-DRB1*11 haplotypes can generate T cells that suppress autoreactive T cells, even those generated by HLA-DRB1*15.

Additionally a strong association of MS and haplotype DRB1* 1501, DQA1*0102, DQB1* 0602 was observed in patients included in the present study (Table 4). This result corresponds to the previous repeatedly confirmed association of HLA class II DRB1* 1501/DQA1*0102, DQB1* 0602 (DR2) region with MS for most Caucasians haplotypes. [35] Dayment *et al*., have been fine mapped this haplotype in the North European population and they have estimated risk ratios of approximately 3, and homozygosity for this haplotype increases MS risk sixfold.[6] Interestingly a risk-associated haplotype to MS in our patient sample was previously identified as protective for T1DM also among Cuban patients,[23] which agrees with other reports of patients with European ancestry suffering from these autoimmune diseases.

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