Journal of Neuroimmunology 330 (2019) 74–80

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Familial multiple sclerosis in a Brazilian sample: Is *HLA-DR15* involved in susceptibility to the disease?

Melina da Silva Bernardes[a,*], Carmen Lucia Antão Paiva[b], Eduardo Ribeiro Paradela[a], Marcos Papais Alvarenga[a,c], Fernanda Ferreira Pereira[a], Claudia Cristina Vasconcelos[a,c], Regina Maria Papais Alvarenga[a,c]

[a] Graduate Progam in Neurology (PPGNEURO), Hospital Universitário Gaffrée e Guinle (HUGG), Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rua Mariz e Barros 775, Rio de Janeiro/RJ 20270-004, Brazil
[b] Department of Genetics and Molecular Biology, Instituto Biomédico, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rua Frei Caneca 94, Rio de Janeiro/RJ 20211-040, Brazil
[c] Department of Neurology, Hospital da Lagoa, Ministry of Health, Rua Jardim Botânico 501, Rio de Janeiro/RJ 22470-050, Brazil

ARTICLE INFO

Keywords:
Familial
Multiple sclerosis
HLA-DR15
HLA-DRB1
Susceptibility
Brazilians

ABSTRACT

Background: The HLA-DR15 extended haplotype *HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02* comprises the strongest genetic risk factor for multiple sclerosis (MS). The aim of this work was to investigate whether HLA-DR15 alleles were significantly associated with the susceptibility to MS familial forms (MSf) in an admixed Brazilian population.
Methods: Association analyses between DR15 and the clinical and demographic variables were made.
Results: We have genotyped 25 familial cases. The DR15 was detected in 11/25 (44%) of them and in none of controls ($P < .00001$). DR15 was significantly associated to a foreign ancestor background ($P = .029$) and later age of onset ($P = .018$).

1. Introduction

Although multiple sclerosis (MS) has a complex and multifactorial etiology, the human leukocyte antigen (HLA) genes have been implicated in susceptibility to the disease for > 40 years (Naito et al., 1972). The HLA-Class II alleles represent independently the major genetic contribution to MS risk, specifically within the DR15 haplotype: *HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02* - which has been a common finding in MS populations, mainly those of Northern European descent (Haines et al., 1998).

Similarly, the association of HLA-DR15 extended or partial haplotypes with the risk of MS was observed in Mediterraneans, (Villoslada et al., 2002; Cocco et al., 2013) Northern Africans (Messadi et al., 2010; Ouadghiri et al., 2013) and Colombians (Rojas et al., 2010). However, in African Americans, Iranians and French Afro-Caribbeans, susceptibility was independently conferred by *DRB1*15:01* genotype (Oksenberg et al., 2004; Ghabaee et al., 2008; Deschamps et al., 2010).

Brazilian studies also have found *DRB1*15:01* participation in MS (Alves-Leon et al., 2007; Paradela et al., 2015).

Relatives of probands are at increased risks of developing MS, since recurrence risk for first-degree relatives even reaches 50 times the rate for the general population (Sadovnick and Baird, 1988). This familial aggregation emphasizes the genetic factor behind MS. Families with two or more cases of MS (familial forms) are suitable models for studying the effect of genetic risk factors (Chao et al., 2009).

The aim of this work was to investigate whether *HLA-DR15* alleles were also significantly associated with the susceptibility to familial forms of MS (MSf) from an admixed Brazilian population - in which African Brazilians constitute 31% of sporadic MS subjects (the remaining 69% were White Brazilians) (Papais-Alvarenga et al., 2015).

2. Patients and methods

We performed a cross-sectional observational study in a reference center for diagnosis and treatment of idiopathic inflammatory demyelinating diseases of the central nervous system (IIDD).

Our institution (Hospital Federal da Lagoa) is a public general hospital, located in Rio de Janeiro city (the second most populous city in the country, counting over 6 million inhabitants) to where the majority (75%) of MS patients, who have been residing in Rio de Janeiro

* Corresponding author.
E-mail address: melina.sbernardes@gmail.com (M. da Silva Bernardes).

https://doi.org/10.1016/j.jneuroim.2019.02.004
Received 3 October 2018; Received in revised form 20 January 2019; Accepted 13 February 2019
0165-5728/ © 2019 Elsevier B.V. All rights reserved.

and adjoining cities, are referred (Papais-Alvarenga et al., 2015).

MS patients followed until 2011 had their diagnosis reviewed by a neurologist team, according to McDonald criteria (Polman et al., 2005). Those patients who had mentioned at least one relative affected by MS were included as MSf in a preliminary selection.

Further interviews, medical record revisions and clinical evaluations were made to refine the sample.

We excluded cases whose relatives could not meet diagnostic criteria by our neurologist team or by external neurologists who had assisted them in other clinical settings. MS affected families whose members had already died, or were unavailable for evaluation, were also excluded from this project. New cases were included until 2014.

Healthy controls' blood samples (N = 116), derived from 70 females (60.3%) and 46 males (39.7%), were obtained for comparison of genotypic frequencies. All controls were natives of Rio de Janeiro state, which encompasses Rio de Janeiro city.

2.1. Variables

Demographic and clinical information of MSf were collected while evaluating patients or reviewing medical records. Age, gender, foreign ancestry (until third-degree ancestor) and outpatient care origin were assessed. Ethnicity was determined by skin color and phenotypic features.

The clinical parameters evaluated were age of onset, disease duration, clinical course and severity. The last, measured by the Multiple Sclerosis Severity Score (MSSS). MSSS is a useful algorithm for comparing disease progression among patients, considering disease duration and disability (Roxburgh et al., 2005). The onset was defined as the time of the first attack - symptoms either registered in medical records or recalled by the patient, which came earlier, as long as compatible with injuries described in Kurtzke Functional Systems (Kurtzke, 1983). Disease courses were defined according to Lublin et al. (2014) revised classification as isolated clinical syndrome (ICS), relapsing-remitting (RRMS), secondary progressive (SPMS) and primary progressive (PPMS).[20]

Families were assorted into 3 groups according to kinship, as proposed by previous studies: first-degree (pairs related by first-degree), multiplex (families with > 3 MSf cases related by any degree), second/third-degrees (pairs related by second-degree or further levels) (Weinshenker et al., 1990). We have also classified the cases regarding generational relationships between the co-affected relatives as: collinear (siblings, cousins) or intergenerational (parents - children; aunt/uncle - niece/nephew).

2.2. HLA genotyping

DNA was isolated from peripheral blood samples by organic method, and the *HLA-DRB1, HLA-DQB1* and *HLA-DQA1* alleles were identified by PCR amplification with sequence-specific primers (SSP-PCR) for typing the HLA alleles, using the One Lambda commercial kit (Canoga Park, CA, USA), according to the manufacturer's recommendations; followed by capillary electrophoresis on the ABI PRISM® 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA) platform to identify the genetic profiles in the patient and control groups. All blood samples were obtained after informed consent. And this study has been approved by Human Research Ethics Committee of the Universidade Federal do Estado do Rio de Janeiro.

2.3. Statistics

The association between categorical variables were analyzed either by Pearson's Chi-square (χ^2) or Fisher's Exact Test (for cell expected count lesser than 5).

For quantitative variables, we chose the non-parametric test: Mann-Whitney U.

Table 1
Familial forms: clinical and demographic data.

	N (valid cases %)	Missing
Sex (F 3.6: M 1)		0
Female	29 (78.4)	
Male	8 (21.6)	
Ethnicity		0
African Brazilians	4 (10.8)	
White	33 (89.2)	
Foreign ancestry	9 (60)	22

	Mean [SD]	Missing
Age	43.1 [± 11.6]	1
Age of onset	28.8 [± 10.6]	1
Disease duration (years)	14.4 [± 9.4]	1
Clinical course	N (%)	0
ICS	0	
RRMS	24 (64.9)	
SPMS	7 (18.9)	
PPMS	6 (16.2)	

	Median (interval[a])	Missing
MSSS[b]	1.98 (0.11–9.95)	2
First-degree	1.64 (0.22–9.58)	1
Multiplex	7.98 (0.67–8.98)	0
Second/third-degrees	2.44 (0.11–9.95)	1

SD: standard deviation.
[a] Minimum and maximum values.
[b] Multiple Sclerosis Severity Score.

The level of statistical significance was set at.05.

Concordance within families was tested by Cohen's *kappa* coefficient (k) for categorical data and by intraclass correlation coefficient (r) for quantitative data.

SPSS 13.0 software package was used for data analysis.

3. Results

We have found 37 eligible MSf cases of a total of 18 families, from which 33 MSf cases (89.2%) were followed by our team. Twenty-nine blood samples were obtained, leading to only 25 successful genotyping results (67.6% of total MSf cases) regarding to 15 families.

In an overall analysis (N = 37 patients), the families were categorized, according to kinship, in: first-degree relative group with 13 families (N = 26); second/third-degree relative group with four families (N = 8); and only one family categorized as multiplex, with a family trio (N = 3).

Demographical and clinical data are summarized in Table 1.

When comparing the genotyped patients (N = 25) with the non-genotyped ones (N = 12) regarding demographic characteristics, no significant differences were observed for age (P = .806), ethnicity (P = .582), foreign ancestry (P = .486) and gender (P = 1.000). The sole difference between these groups resided in the outpatient care origin (P = .007), once eight from the twelve non-genotyped patients were clinically attended in other institutions.

The majority of MSf cases (N = 27; 73%) consisted of collinears. Intergenerational pairs represented a smaller proportion (N = 10; 27%).

3.1. HLA - DR15 alleles

The extended *DR15* haplotype was found in 11/25 patients (44%). Other six patients (24%) had a pair of distinct alleles of *DR15* haplotype - *DRB1*15:01*, *DQA1*01:02* or *DQB1*06:02*. Three patients (12%) carried only one distinct allele of DR15, while five patients (20%) did

Table 2
*DRB1*15:01*, *DQA1*01:02* and *DQB1*06:02* alleles within the families.

			Course	*DRB1*15:01*	*DQA1*01:02*	*DQB1*06:02*
First-degree	Family 1	Sister	RRMS	+	+	+
		Sister	RRMS	+	+	-
	Family 2	Brother	RRMS	−	−	−
	Family 3	Sister	PPMS	+	+	+
		Sister	PPMS	+	+	+
	Family 4	Sister	RRMS	−	−	+
		Sister	RRMS	−	−	+
	Family 5	Sister	RRMS	+	+	+
		Sister	RRMS	+	+	+
	Family 6	Sister	SPMS	−	+	−
		Brother	RRMS	−	−	−
	Family 7	Brother	RRMS	+	+	+
	Family 8	Mother	PPMS	−	−	−
	Family 9	Mother	SPMS	−	+	+
		Daughter	RRMS	−	+	+
	Family 10	Brother	RRMS	+	−	+
Multiplex	Family 11	Sister	PPMS	+	+	+
		Sister	PPMS	+	+	+
		Sister	RRMS	+	+	+
Second/third-degrees	Family 12	Cousin[a]	RRMS	+	+	+
		Cousin[a]	RRMS	+	+	+
	Family 13	Cousin[a]	SPMS	−	−	−
		Cousin[b]	SPMS	−	−	−
	Family 14	Niece	RRMS	−	+	+
	Family 15	Niece	RRMS	−	+	+

PPMS: primary progressive multiple sclerosis. RRMS: relapsing-remitting multiple sclerosis. SPMS: secondary progressive multiple sclerosis.
[a] Female.
[b] Male.

not show any allele of this haplotype. The detailed results are displayed on Table 2. When considering those alleles separately, the *DQB1*06:02* was the most frequent, carried by 18 patients (72%). *DQB1*06:02* was also the sole *DR15* allele found in the only two genotyped African Brazilians from this sample – a pair of sisters. The second most frequent allele was *DQA1*01:02*, found in 17 cases (68%). *DRB1*15:01* was detected in 13 individuals (52%) and each one of them, with the exception of two patients, also had the extended haplotype.

Distinguishing patients by *HLA-DR15* carrier status, we found that the HLA-DR15 positive result was statistically significant when associated with foreign ancestor background (P = .029) and later age of onset (P = .017). After multivariate analysis, these variables proved to be independently associated with *HLA-DR15*. It was noted a tendency of association of PPMS with *HLA-DR15* (P = .084). All intergenerational cases tested were *HLA-DR15* negative, and this association was statistically significant (P = .046) after the scrutiny of multivariate analysis. The female-to-male ratio was higher for the HLA-DR15 positive group (female: male = 10) compared with HLA-DR15 negatives (female: male = 2.5), although the difference was found not significant (P = .341). No statistically significant differences were found between the HLA-DR15 carriers and non-carriers regarding ethnicity or disease severity (Table 3).

The extended HLA-DR15 was not observed in the controls. This strongly contrasted with MSf results; furthermore, the difference between the two groups is of statistical significance (*HLA-DR15* frequencies: patients - 44% vs. controls - 0%; two-tailed P < .00001). Considering each allele frequency independently, all of the three alleles were overrepresented in MSf cases when comparing with controls (Table 4). However, after applying Bonferroni correction for 83 variables (all *DRB1*, *DQB1* and *DQA1* alleles) analysis, the new P value set was .0006 and *DQA1*01:02* association lost statistical significance.

4. Discussion

MS familial forms account for various proportions of overall MS cases around the world and might be as frequent as one familial case in five (20:100) of MS cases among Caucasians from North America (Ebers et al., 2000). The only published study regarding epidemiology of MSf in Rio de Janeiro, Brazil, found a lower prevalence rate of 6.12:100 (Papais-Alvarenga et al., 2015).

HLA-DR15 haplotype or *DRB1*15:01* allele alone have been associated with MSf in many studies including populations of Caucasian descent (Fogdell et al., 1997; Haines et al., 1998; The Multiple Sclerosis Genetics Group et al., 2002).

One study with Latins had provided data regarding HLA Class II alleles in MSf. Alvarado-de la Barrera et al. (2000) had investigated the occurrence of *DRB1*15:01* and other *HLA-DRB1*, *DQA1* and *DQB1* alleles among Mexican Mestizos affected by MS. Eight patients with sporadic MS and other nine patients with MSf (all Mexican mestizos) were genotyped. Although not relied on statistical analysis matching controls, they found that the most frequent *DRB1* alleles for MS patients were *DR15* (*15:01) and *DR3* (*03:01).

Albeit not performed with familial forms, other studies in Latin American populations have investigated the influence of *DRB1*, *DQA1*, *DQB1* alleles on susceptibility to MS in the past two decades (Tables 5–6). The most frequent finding is the risk attributable to *DRB1*15:01*; however, distinct Class II alleles were implicated in the susceptibility to MS depending on the particular ethnic background of the populations. The different patterns of linkage disequilibrium (LD) seen in those admixed populations favors the estimation of the risk contribution of a single allele.

In Brazilians, the investigations of *HLA Class II alleles* in MS have been restricted to samples from the Southeast and the South Regions – which have the greatest proportions of White population in the country. The Southeast had been the political center of the Colony since late XVIII century, and in 1808 the Portuguese court moved entirely there. This region attracted many Europeans from Iberian Peninsula; on the other hand, many Africans were brought to it by the force of slavery for four centuries. Unlike that, the South Region had not had an important role in the influx of African slaves. Moreover, in the XX century many Europeans (mainly Italians and Germans) immigrants settled there impelled by The Great Wars.

Table 3
Comparisons between groups defined by *HLA-DR15* status.

(N)		*HLA-DR15* positive	*HLA-DR15* negative	P value for comparisons
Sex (25)	Female	10	10	.341[a]
	Male	1	4	
Ethnicity (22)	Afro	0	2	.487[a]
	White	11	12	
Foreign ancestor (13)	Positive	6	1	**.029**[a]
	Negative	1	5	
Kinship groups (22)	First-degree	6	10	.135[a]
	Multiplex	3	0	
	Second/third-degrees	2	4	
Generations (22)	Intergenerational	0	5	**.046**[a]
	Collinear	11	9	
Clinical course (22)	RRMS	7	9	.084[a]
	SPMS	0	4	
	PPMS	4	1	
Age of onset - years (22)	29.76 ± 10.34[*]	35.27 ± 10.34[*]	25.43 ± 8.32[*]	**.017**[b]
MSSS (22)	2.44 (0.11–9.95)[†]	2.64 (0.11–8.98)[†]	2.44 (0.24–9.95)[†]	.848[b]

In bold: Statistically significant results.
[a] Two-sided probability values calculated after Fisher's Exact Test.
[b] Two-tailed probability values calculated after Mann-Whitney Test.
[*] Mean ± standard deviation.
[†] Median (minimum - maximum).

Hereupon, the major studies on *HLA* class II alleles and MS performed for Southeast Region had attributed risk of MS to one or more alleles belonging to either of these *HLA* regions: *DRB1*, *DQB1* and *DQA1*.

Alves-Leon et al. (2007) found that *DRB1*15:01* conferred risk for MS in Whites, whereas *DQB1*06:02* was implied in risk for African Brazilians. Noteworthy, *DQA1*02:01* was associated to risk of MS in African Brazilians, in spite of this allele be significantly more frequent in healthy White individuals. Brum et al. (2007) not only confirmed the *DRB1*15:01* association to MS in White individuals, but also proved susceptibility to MS in African Brazilians (Mulattos) carrying *DRB1*15:03*. Similarly, we have observed a high frequency of *DRB1*15:01* in our predominantly White sample. Indeed, the African Brazilians genotyped here had the *DQB1*06:02* allele and were *DRB1*15:01* negative. Additionally, it is worth mentioning that our tested sample had a lower proportion of African Brazilians compared with that observed for sporadic MS (4.5% vs. 31%) (Papais-Alvarenga et al., 2015). The significant association of foreign ancestor background and *HLA-DR15* carrier status we have observed point towards the European genetic pattern inherited by the probands.

In a more recent analysis, many other alleles were associated to MS susceptibility: *DRB1*11:01/13:02/13:03/**15:01***, *DQA1*05:01/3/5*, *DQB1*03:01/**06:02**/06:04*. (Alvarenga et al., 2017). However those results were not stratified according to ethnicity.

In contrast, in South Region only the *DQB1*02:03* was significantly associated with MS after Bonferroni correction of probability considering the number of analyzed alleles (Werneck et al., 2016). No *DRB1* or other *DQB1* alleles were found in significant greater frequencies among MS patients.

Few studies with familial forms have investigated HLA-DR15 influence on clinical and demographic characteristics. The Multiple Sclerosis Genetics Group showed no significant association of HLA-DR15 with age of onset (The Multiple Sclerosis Genetics Group et al., 2002). Oturai et al. (2004) reported early age of onset associated with *HLA-DR15*. On the other hand, we had demonstrated that *HLA-DR15* carriers exhibited later age of onset. This finding agrees with an increased frequency of primary progressive course among those cases. Interestingly, other Brazilian study had previously shown association of sporadic PPMS with *DRB1*15:01* and *DQB1*06:02* irrespective of ethnicity (Vasconcelos et al., 2009).

Data about disease severity are likewise scarce. A study from Canary Islands shows that MS familial forms had more benign course compared to MS sporadic cases (Amela-Peris et al., 2004). The analysis was not stratified according to *HLA-DR15* status - although more than half of the familial forms had the *HLA-DRB1*1501* allele. There was no statistical discrepancy between HLA-DR15 carriers and non-carriers regarding MSSS in our sample. Our results agree with those found by The Multiple Sclerosis Genetics Group regardless of the fact that they used EDSS (Expanded Disability Status Scale) categories for measuring disease severity (The Multiple Sclerosis Genetics Group et al., 2002).

Regarding gender, *HLA-DRB1*15:01* was previously associated with risk to MS and this risk was restricted to females. This was found in studies involving Spanish and Brazilian populations (Irizar et al., 2011; Paradela et al., 2015). In our study, the female-to-male ratio was higher among *HLA-DR15* positive patients compared to *HLA-DR15* negatives, although the difference was not statistically significant. This could reflect the *HLA-DRB1*1501* influence, since this allele was frequently present together with *DQA1*01:02* and *DQB1*06:02* in our sample of MSf patients.

Interestingly, none of our intergenerational cases had the *HLA-DR15* haplotype. However, Chao et al. have indicated that *HLA-DBR1*1501* confers a greater risk among intergenerational second-degree pairs compared to collinear (siblings) pairs (Chao et al., 2008).

Even in our familial cases, the strongest genetic risk factor – the *HLA-DR15* alleles - could not be traced in half of the genotyped cases. While influence of genetics on MS is supported by high recurrence risks among relatives, the trait does not result from a mendelian pattern, nor depends on few alleles with large effects. The role of polymorphic variants outside the HLA regions and polygenic interactions of modest effects have become evident in genome-wide association studies (International Multiple Sclerosis Genetics Consortium et al., 2007; Sawcer et al., 2011). Their findings have partially elucidated the recurrence risks found in HLA-DR15 negative patients.

Finally, although, our statistical results are affected by our restricted sample size, our sample is very representative of thoroughly registered population of hundreds of MS cases.

5. Conclusions

We have investigated HLA-DR15 influence on MS familial forms among Brazilians from Rio de Janeiro State. In our study, *HLA-DR15* was strongly associated to MS familial cases compared to healthy controls. Also, *HLA-DR15* positive patients had later age of onset of the disease and reported more often as having a foreign (Caucasian

Table 4
(A) *DRB1*, (B) *DQB1* and (C) *DQA1* allele frequencies for MSf and controls.

Allele	MSf	Controls	P value[b]
	N = 25[a]	N = 116[a]	
A - Frequencies of *HLA-DRB1* alleles for patients and controls.			
*01:01	2 (0.040)	9 (0.039)	1.000
*01:02	0	4 (0.017)	.345
*01:03	0	8 (0.034)	.163
*03:01	4 (0.080)	13 (0.056)	.211
*03:02	0	4 (0.017)	.345
*03:05	1 (0.020)	4 (0.017)	.429
*03:08	0	3 (0.013)	1.000
*03:11	0	3 (0.013)	1.000
*04:01	0	2 (0.009)	1.000
*04:02	1 (0.020)	3 (0.013)	.546
*04:03	1 (0.020)	3 (0.013)	.546
*04:04	0	1 (0.004)	1.000
*04:05	1 (0.020)	3 (0.013)	.546
*04:06	0	1 (0.004)	1.000
*04:07	0	3 (0.013)	1.000
*04:08	0	1 (0.004)	1.000
*04:09	0	3 (0.013)	1.000
*04:10	0	4 (0.017)	1.000
*04:11	0	7 (0.030)	.353
*07:01	3 (0.060)	9 (0.039)	.446
*08:01	1 (0.020)	8 (0.034)	1.000
*08:03	0	5 (0.022)	.586
*08:04	0	1 (0.004)	1.000
*08:07	0	3 (0.013)	1.000
*09:01	0	2 (0.009)	1.000
*10:01	0	1 (0.004)	1.000
*11:01	7 (0.140)	9 (0.039)	.009
*11:02	0	9 (0.039)	.361
*11:03	0	2 (0.009)	1.000
*11:04	3 (0.060)	1 (0.004)	.018
*12:01	1 (0.020)	2 (0.009)	.446
*12:02	0	2 (0.009)	1.000
*13:01	1 (0.020)	10 (0.043)	.689
*13:02	0	3 (0.013)	1.000
*13:03	4 (0.080)	8 (0.034)	.226
*13:06	0	3 (0.013)	1.000
*13:09	0	2 (0.009)	1.000
*14:01	0	2 (0.009)	1.000
*14:02	0	4 (0.017)	1.000
*14:05	0	1 (0.004)	1.000
*14:06	0	2 (0.009)	1.000
***15:01**	**13 (0.260)**	**13 (0.056)**	**< .0001**
*15:02	0	12 (0.052)	.125
*15:03	0	10 (0.043)	.209
*16:01	0	6 (0.026)	.591
*16:02	2 (0.040)	13 (0.056)	1.000
*16:03	1 (0.020)	10 (0.043)	.689
B – Frequencies of *HLA-DQB1* alleles for patients and controls			
*02:01	7 (0.140)	33 (0.142)	.964
*02:02	3 (0.60)	0	.005
*02:03	0	4 (0.017)	1.000
*03:01	4 (0.080)	16 (0.069)	.756
*03:02	6 (0.120)	17 (0.073)	.246
*03:03	0	8 (0.034)	.351
*03:04	0	6 (0.026)	.591
*03:05	0	1 (0.004)	1.000
*03:07	0	2 (0.009)	1.000
*04:01	3 (0.060)	14 (0.060)	1.000
*04:02	0	5 (0.022)	.586
*05:01	3 (0.060)	33 (0.142)	.087
*05:02	0	9 (0.039)	.361
*05:03	2 (0.040)	16 (0.069)	.741
*05:04	0	3 (0.013)	1.000
*06:01	0	6 (0.026)	.591
***06:02**	**18 (0.360)**	**19 (0.082)**	**< .0001**
*06:03	2 (0.040)	13 (0.056)	1.000
*06:04	0	4 (0.017)	1.000
*06:05	0	3 (0.013)	1.000
*06:06	0	4 (0.017)	1.000
*06:07	1 (0.020)	5 (0.022)	1.000
*06:08	0	9 (0.039)	.361
*06:09	0	0	–
*06:10	0	0	–
*06:11	0	2 (0.009)	1.000
C – Frequencies of *HLA-DQA1* alleles for patients and controls.			
*01:01	4 (0.080)	27 (0.116)	.426
*01:02	17 (0.340)	39 (0.168)	.001
*01:03	0	19 (0.082)	.025
*01:04	2 (0.040)	11 (0.047)	1.000
*02:01	7 (0.140)	38 (0.164)	.643
*03:01	4 (0.080)	36 (0.155)	.130
*04:01	3 (0.060)	14 (0.060)	1.000
*05:01	8 (0.160)	33 (0.142)	.723
*05:02	2 (0.040)	14 (0.060)	.737
*05:05	3 (0.060)	0	.005
*06:01	0	1 (0.004)	1.000
*06:02	1 (0.020)	0	.177

MSf: multiple sclerosis familial forms.
In bold: results statistically significant after Bonferroni correction.
[a] Allelic frequency.
[b] Two-tailed probability values calculated after Chi-square Test or Fisher's Exact Test.

Table 5
Frequencies of the alleles *HLA-DRB1*15:01*, *DQA1*01:02*, *DQB1*06:02* for chromosomes (2N): data from Latin American studies for MS patients and controls.

Study	Patients	DRB1*	DQA1*	DQB1*
Population	(N)	15:01[a] (n)	01:02[a] (n)	06:02[a] (n)
Alvarado-de la Barrera et al. (2000)	MSf	9	0.222 (4)	0.111 (2)	0.111 (2)
	Sporadic MS	8	0.250 (4)	0.250 (4)	0.250 (4)
Mexican Mestizos	Controls	99	0.045 (9)	0.085 (17)	0.075 (15)
Alaez et al. (2005)	Sporadic MS	51	0.029 (3)	0.049 (5)	0.049 (5)
Mexican Mestizos	Controls	173	0.046 (16)	0.090 (31)	0.058 (20)
Rojas et al. (2010)	Sporadic MS	65	0.062	–	–
Paisa Community[b]	Controls	184	0.033	–	–
Werneck et al. (2016)	Sporadic MS	86	0.140 (24)	–	–
	Controls	606	0.081 (98)	–	–
Admixed; mainly White Brazilians	Sporadic MS	85	–	–	0.171 (29)
	Controls	220	–	–	0.125 (55)
Our sample	MSf	22	0.295 (13)	0.386 (17)	0.409 (18)
Admixed; mainly White Brazilians	Controls	116	0.056 (13)	0.168 (39)	0.082 (19)

N: number of individuals. MSf: multiple sclerosis familial forms. n: number of alleles.
[a] Allele frequencies.
[b] A distinct population residing in Medellin (Antioquia, Colombia) genetically characterized as a Caucasoid group (Bravo et al., 1996).

European) ancestor. There was a tendency for PPMS course among *HLA-DR15* positive patients.

Declaration of conflicting interests

The authors declare that there is no conflict of interest to disclose.

Acknowledgements

We thank the Prof. Ronir Raggio Luiz, from Universidade Federal do

Table 6
Phenotypic frequencies (i.e., number of individuals carrying at least one allele copy) of the alleles *HLA-DRB1*15:01*, *DQA1*01:02*, *DQB1*06:02*: data from Latin American studies for MS patients and controls.

Study		Patients (N)	*DRB1**	*DQA1**	*DQB1**
Population			*15:01* (n)	*01:02* (n)	*06:02* (n)
Quelvennec et al. (2003)	Sporadic MS	55	0.110	–	0.490
Admixed Martinicans	Controls	100	0.030	–	0.290
Alves-Leon et al. (2007)	Sporadic MS	84	0.226 (19)	0.429 (36)	0.405(34)
Admixed Brazilians	Controls	180	0.067 (12)	0.372 (67)	0.150 (27)
Brum et al. (2007)	Sporadic MS	113	0.327 (37)	–	–
Admixed Brazilians	Controls	114	0.140 (16)	–	–
Vasconcelos et al. (2009)	Sporadic MS	33	0.182 (12)	0.227 (15)	0.212 (14)
Admixed Brazilians	Controls	180	0.033 (12)	0.186 (67)	0.075 (27)
Alvarenga et al. (2017)	Sporadic MS	94	0.277 (26)	0.489 (46)	0.394 (37)
Admixed Brazilians	Controls	100	0.080 (8)	0.380 (38)	0.180 (18)

N: number of individuals. n: number of individuals carrying the allele.

Rio de Janeiro (UFRJ), for providing statistical assistance on writing this manuscript.

Funding

This research was supported by UNIRIO (Universidade Federal do Estado do Rio de Janeiro) and CAPES/PROAP.

References

Alaez, C., Corona, T., Ruano, L., Flores, H., Loyola, M., Gorodezky, C., 2005. Mediterranean and Amerindian MHC class II alleles are associated with multiple sclerosis in Mexicans. Acta Neurol. Scand. 112 (5), 317–322.

Alvarado-de la Barrera, C., Zuniga-Ramos, J., Ruiz-Morales, J.A., Estanol, B., Granados, J., Llorente, L., 2000. HLA class II genotypes in Mexican Mestizos with familial and nonfamilial multiple sclerosis. Neurology 55 (12), 1897–1900.

Alvarenga, M.P., Fernandez, O., Leyva, L., Campanella, L., Vasconcelos, C.F., Alvarenga, M., Papais Alvarenga, R.M., 2017. The HLA DRB1*03:01 allele is associated with NMO regardless of the NMO-IgG status in Brazilian patients from Rio de Janeiro. J. Neuroimmunol. 310, 1–7.

Alves-Leon, S.V., Papais-Alvarenga, R., Magalhães, M., Alvarenga, M., Thuler, L.C., Fernández y Fernandez, O., 2007. Ethnicity-dependent association of HLA DRB1-DQA1-DQB1 alleles in Brazilian multiple sclerosis patients. Acta Neurol. Scand. 115 (5), 306–311.

Amela-Peris, R., Aladro, Y., Conde-Sendín, M.A., Alemany-Rodríguez, M.J., Muñoz-Fernández, C., Reyes-Yáñez, M.P., Déniz-Naranjo, M.C., Sánchez-García, F., 2004. Rev. Neurol. 39 (10), 911–914 Familial multiple sclerosis in Canary Islands]. [Article in Spanish.

Bravo, M.L., Valenzuela, C.Y., Arcos-Burgos, O.M., 1996. Polymorphisms and phyletic relationships of the Paisa community from Antioquia (Colombia). Gene Geogr. 10 (1), 11–17.

Brum, D.G., Barreira, A.A., Louzada-Junior, P., Mendes-Junior, C.T., Donadi, E.A., 2007. Association of the HLA-DRB1N15 allele group and the DRB1N1501 and DRB1N1503 alleles with multiple sclerosis in White and Mulatto samples from Brazil. J. Neuroimmunol. 189 (1–2), 118–124.

Chao, M.J., Ramagopalan, S.V., Herrera, B.M., Lincoln, M.R., Dyment, D.A., Sadovnick, A.D., Ebers, G.C., 2008. Epigenetics in multiple sclerosis susceptibility: difference in transgenerational risk localizes to the major histocompatibility complex. Hum. Mol. Genet. 18 (2), 261–266.

Chao, M.J., Ramagopalan, S.V., Herrera, B.M., Lincoln, M.R., Dyment, D.A., Sadovnick, A.D., Ebers, G.C., 2009. Epigenetics in multiple sclerosis susceptibility: difference in transgenerational risk localizes to the major histocompatibility complex. Hum. Mol. Genet. 18 (2), 261–266.

Cocco, E., Murru, R., Costa, G., Kumar, A., Pieroni, E., Melis, C., Barberini, L., Sardu, C., Lorefice, L., Fenu, G., Frau, J., Coghe, G., Carboni, N., Marrosu, M.G., 2013. Interaction between HLA-DRB1-DQB1 Haplotypes in sardinian Multiple Sclerosis Population. PLoS One 8 (4), e59790.

Deschamps, R., Paturel, L., Jeannin, S., Chausson, N., Olindo, S., Béra, O., Bellance, R., Smadja, D., Césaire, D., Cabre, P., 2010. Different HLA class II (DRB1 and DQB1) alleles determine either susceptibility or resistance to NMO and multiple sclerosis among the French Afro-Caribbean population. Mult. Scler. J. 17 (1), 24–31.

Ebers, G., Koopman, W., Hader, W., Sadovnick, A., Kremenchutzky, M., Mandalfino, P., Wingerchuk, D., Baskerville, J., Rice, G., 2000. The natural history of multiple sclerosis: a geographically based study: 8: familial multiple sclerosis. Brain 123 (3), 641–649.

Fogdell, A., Olerup, O., Fredrikson, S., Vrethem, M., Hillert, J., 1997. Linkage analysis of HLA class II Genes in Swedish multiplex families with multiple sclerosis. Neurology 48 (3), 758–762.

Ghabaee, M., Bayati, A., Amri Saroukolaei, S., Sahraian, M.A., Sanaati, M.H., Karimi, P., Houshmand, M., Sadeghian, H., Hashemi Chelavi, L., 2008. Analysis of HLA DR2& DQ6 (DRB1*1501, DQA1*0102, DQB1*0602) haplotypes in Iranian patients with multiple sclerosis. Cell. Mol. Neurobiol. 29 (1), 109–114.

Haines, J.L., Terwedow, H.A., Burgess, K., Pericak-Vance, M.A., Rimmler, J.B., Martin, E.R., Oksenberg, J.R., Lincoln, R., Zhang, D.Y., Banatao, D.R., Gatto, N., Goodkin, D.E., Hauser, S.L., 1998. The Multiple Sclerosis Genetics Group. Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity. Hum. Mol. Genet. 7 (8), 1229–1234.

International Multiple Sclerosis Genetics Consortium, Hafler, D.A., Compston, A., Sawcer, S., Lander, E.S., Daly, M.J., De Jager, P.L., de Bakker, P.I., Gabriel, S.B., Mirel, D.B., Ivinson, A.J., Pericak-Vance, M.A., Gregory, S.G., Rioux, J.D., McCauley, J.L., Haines, J.L., Barcellos, L.F., Cree, B., Oksenberg, J.R., Hauser, S.L., 2007. Risk alleles for multiple sclerosis identified by a genomewide study. N. Engl. J. Med. 357 (9), 851–862.

Irizar, H., Muñoz-Culla, M., Zuriarrain, O., Goyenechea, E., Castillo-Triviño, T., Prada, A., Saenz-Cuesta, M., De Juan, D., Lopez de Munain, A., Olascoaga, J., Otaegui, D., 2011. HLA-DRB1*15:01 and multiple sclerosis: a female association. Mult. Scler. J. 18 (5), 569–577.

Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33 (11), 1444.

Lublin, F.D., Reingold, S.C., Cohen, J.A., Cutter, G.R., Sorensen, P.S., Thompson, A.J., Wolinsky, J.S., Balcer, L.J., Banwell, B., Barkhof, F., Bebo, B., Calabresi, P.A., Clanet, M., Comi, G., Fox, R.J., Freedman, M.S., Goodman, A.D., Inglese, M., Kappos, L., Kieseier, B.C., Lincoln, J.A., Lubetzki, C., Miller, A.E., Montalban, X., O'Connor, P.W., Petkau, J., Pozzilli, C., Rudick, R.A., Sormani, M.P., Stuve, O., Waubant, E., Polman, C.H., 2014. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology 83 (3), 278–286.

Messadi, A., Najiba, F.M., Ouerhani, S., Zaweli, J., Louatti, I., Layouni, S., Nciri, B., Bouaicha, G., Kouki, W., Yedeas, M., Raies, A., Mrissa, R., Gritli, N., 2010. HLA class II alleles and multiple sclerosis in Tunisian patients. Clin. Neurol. Neurosurg. 112 (10), 849–852.

Naito, S., Namerow, N., Mickey, M.R., Terasaki, P.I., 1972. Multiple sclerosis: association with HL—A3. Tissue Antigens 2, 1–4. https://doi.org/10.1111/j.1399-0039.1972.tb00111.x.

Oksenberg, J.R., Barcellos, L.F., Cree, B.A., Baranzini, S.E., Bugawan, T.L., Khan, O., Lincoln, R.R., Swerdlin, A., Mignot, E., Lin, L., Goodin, D., Erlich, H.A., Schmidt, S., Thomson, G., Reich, D.E., Pericak-Vance, M.A., Haines, J.L., Hauser, S.L., 2004. Mapping multiple sclerosis susceptibility to the HLA-DR locus in African Americans. Am. J. Hum. Genet. 74 (1), 160–167.

Oturai, A.B., Ryder, L.P., Fredrikson, S., Myhr, K.M., Celius, E.G., Harbo, H.F., Andersen, O., Akesson, E., Hillert, J., Madsen, H.O., Nyland, H., Spurkland, A., Datta, P., Svejgaard, A., Sorensen, P.S., 2004. Concordance for disease course and age of onset in Scandinavian multiple sclerosis coaffected sib pairs. Mult. Scler. 10 (1), 5–8.

Ouadghiri, S., El Alaoui Toussi, K., Brick, C., Ait Benhaddou, E.H., Benseffaj, N., Benomar, A., El Yahyaoui, M., Essakalli, M., 2013. Genetic factors and multiple sclerosis in the Moroccan population: a role for HLA class II. Pathol. Biol. 61 (6), 259–263.

Papais-Alvarenga, R.M., Pereira, F.F., Bernardes, M.S., Papais-Alvarenga, M., Batista, E., Paiva, C.A., Santos, C.M., Vasconcelos, C.C., 2015. Familial forms of multiple sclerosis and neuromyelitis optica at an MS center in Rio de Janeiro State, Brazil. J. Neurol. Sci. 356 (1–2), 196–201.

Paradela, E.R., Alves-Leon, S.V., Figueiredo, A.L., Pereira, V.C., Malfetano, F., Mansur, L.F., Scherpenhuijzen, S., Agostinho, L.A., Rocha, C.F., Rueda-Lopes, F., Gasparetto, E., Paiva, C.L., 2015. The CIITA genetic polymorphism rs4774*C in combination with the HLA-DRB1*15:01 allele as a putative susceptibility factor to multiple sclerosis in Brazilian females. Arq. Neuropsiquiatr. 73 (4), 283–288.

Polman, C.H., Reingold, S.C., Edan, G., Filippi, M., Hartung, H.P., Kappos, L., Lublin, F.D., Metz, L.M., McFarland, H.F., O'Connor, P.W., Sandberg-Wollheim, M., Thompson, A.J., Weinshenker, B.G., Wolinsky, J.S., 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann. Neurol. 58 (6), 840–846.

Quelvennec, E., Bera, O., Cabre, P., Alizadeh, M., Smadja, D., Jugde, F., Edan, G., Semana, G., 2003. Genetic and functional studies in multiple sclerosis patients from Martinique attest for a specific and direct role of the HLA-DR locus in the syndrome. Tissue Antigens 61 (2), 166–171.

Rojas, O.L., Rojas-Villarraga, A., Cruz-Tapias, P., Sánchez, J.L., Suárez-Escudero, J.C.,

Patarroyo, M.A., Anaya, J.M., 2010. HLA class II polymorphism in Latin American patients with multiple sclerosis. Autoimmun. Rev. 9 (6), 407–413.
Roxburgh, R.H., Seaman, S.R., Masterman, T., Hensiek, A.E., Sawcer, S., Vukusic, S., Achiti, I., Confavreux, C., Coustans, M., le Page, E., Edan, G., McDonnell, G.V., Hawkins, S., Trojano, M., Liguori, M., Cocco, E., Marrosu, M.G., Tesser, F., Leone, M.A., Weber, A., Zipp, F., Miterski, B., Epplen, J.T., Oturai, A., Sorensen, P.S., Celius, E.G., Lara, N.T., Montalban, X., Villoslada, P., Silva, A.M., Marta, M., Leite, I., Dubois, B., Rubio, J., Butzkueven, H., Kilpatrick, T., Mycko, M.P., Selmaj, K.W., Rio, M.E., Sá, M., Salemi, G., Savettieri, G., Hillert, J., Compston, D.A., 2005. Multiple Sclerosis Severity Score: using disability and disease duration to rate disease severity. Neurology 64 (7), 1144–1151.
Sadovnick, A.D., Baird, P.A., 1988. The familial nature of multiple sclerosis: age-corrected empiric recurrence risks for children and siblings of patients. Neurology 38 (6), 990.
Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C.C., Patsopoulos, N.A., Moutsianas, L., Dilthey, A., Su, Z., Freeman, C., Hunt, S.E., Edkins, S., Gray, E., Booth, D.R., Potter, S.C., Goris, A., Band, G., Oturai, A.B., Strange, A., Saarela, J., Bellenguez, C., Fontaine, B., Gillman, M., Hemmer, B., Gwilliam, R., Zipp, F., Jayakumar, A., Martin, R., Leslie, S., Hawkins, S., Giannoulatou, E., D'alfonso, S., Blackburn, H., Martinelli Boneschi, F., Liddle, J., Harbo, H.F., Perez, M.L., Spurkland, A., Waller, M.J., Mycko, M.P., Ricketts, M., Comabella, M., Hammond, N., Kockum, I., McCann, O.T., Ban, M., Whittaker, P., Kemppinen, A., Weston, P., Hawkins, C., Widaa, S., Zajicek, J., Dronov, S., Robertson, N., Bumpstead, S.J., Barcellos, L.F., Ravindrarajah, R., Abraham, R., Alfredsson, L., Ardlie, K., Aubin, C., Baker, A., Baker, K., Baranzini, S.E., Bergamaschi, L., Bergamaschi, R., Bernstein, A., Berthele, A., Boggild, M., Bradfield, J.P., Brassat, D., Broadley, S.A., Buck, D., Butzkueven, H., Capra, R., Carroll, W.M., Cavalla, P., Celius, E.G., Cepok, S., Chiavacci, R., Clerget-Darpoux, F., Clysters, K., Comi, G., Cossburn, M., Cournu-Rebeix, I., Cox, M.B., Cozen, W., Cree, B.A., Cross, A.H., Cusi, D., Daly, M.J., Davis, E., de Bakker, P.I., Debouverie, M., D'hooghe, M.B., Dixon, K., Dobosi, R., Dubois, B., Ellinghaus, D., Elovaara, I., Esposito, F., Fontenille, C., Foote, S., Franke, A., Galimberti, D., Ghezzi, A., Glessner, J., Gomez, R., Gout, O., Graham, C., Grant, S.F., Guerini, F.R., Hakonarson, H., Hall, P., Hamsten, A., Hartung, H.P., Heard, R.N., Heath, S., Hobart, J., Hoshi, M., Infante-Duarte, C., Ingram, G., Ingram, W., Islam, T., Jagodic, M., Kabesch, M., Kermode, A.G., Kilpatrick, T.J., Kim, C., Klopp, N., Koivisto, K., Larsson, M., Lathrop, M., Lechner-Scott, J.S., Leone, M.A., Leppä, V., Liljedahl, U., Bomfim, I.L., Lincoln, R.R., Link, J., Liu, J., Lorentzen, Å.R., Lupoli, S., Macciardi, F., Mack, T., Marriott, M., Martinelli, V., Mason, D., McCauley, J.L., Mentch, F., Mero, I.L., Mihalova, T., Montalban, X., Mottershead, J., Myhr, K.M., Naldi, P., Ollier, W., Page, A., Palotie, A., Pelletier, J., Piccio, L., Pickersgill, T., Piehl, F., Pobywajlo, S., Quach, H.L., Ramsay, P.P., Reunanen, M., Reynolds, R., Rioux, J.D., Rodegher, M., Roesner, S., Rubio, J.P., Rückert, I.M., Salvetti, M., Salvi, E., Santaniello, A., Schaefer, C.A., Schreiber, S., Schulze, C., Scott, R.J., Sellebjerg, F., Selmaj, K.W., Sexton, D., Shen, L., Simms-Acuna, B., Skidmore, S., Sleiman, P.M., Smestad, C., Sørensen, P.S., Søndergaard, H.B., Stankovich, J., Strange, R.C., Sulonen, A.M., Sundqvist, E., Syvänen, A.C., Taddeo, F., Taylor, B., Blackwell, J.M., Tienari, P., Bramon, E., Tourbah, A., Brown, M.A., Tronczynska, E., Casas, J.P., Tubridy, N., Corvin, A., Vickery, J., Jankowski, J., Villoslada, P., Markus, H.S., Wang, K., Mathew, C.G., Wason, J., Palmer, C.N., Wichmann, H.E., Plomin, R., Willoughby, E., Rautanen, A., Winkelmann, J., Wittig, M., Trembath, R.C., Yaouanq, J., Viswanathan, A.C., Zhang, H., Wood, N.W., Zuvich, R., Deloukas, P., Langford, C., Duncanson, A., Oksenberg, J.R., Pericak-Vance, M.A., Haines, J.L., Olsson, T., Hillert, J., Ivinson, A.J., De Jager, P.L., Peltonen, L., Stewart, G.J., Hafler, D.A., Hauser, S.L., McVean, G., Donnelly, P., Compston, A., 2011. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 476 (7359), 214–219.
The Multiple Sclerosis Genetics Group, Barcellos, L., Oksenberg, J., Green, A., Bucher, P., Rimmler, J., Schmidt, S., Garcia, M., Lincoln, R., Pericak-Vance, M., Haines, J., Hauser, S., 2002. Genetic basis for clinical expression in multiple sclerosis. Brain 125 (1), 150–158.
Vasconcelos, C.C., Fernández, O., Leyva, L., Thuler, L.C., Alvarenga, R.M., 2009. Does the DRB1$_*$1501 allele confer more severe and faster progression in primary progressive multiple sclerosis patients?HLA in primary progressive multiple sclerosis. J. Neuroimmunol. 214 (1–2), 101–103.
Villoslada, P., Barcellos, L.F., Rio, J., Begovich, A.B., Tintore, M., Sastre-Garriga, J., Baranzini, S.E., Casquero, P., Hauser, S.L., Montalban, X., Oksenberg, J.R., 2002. The HLA locus and multiple sclerosis in Spain. Role in disease susceptibility, clinical course and response to interferon-β. J. Neuroimmunol. 130 (1–2), 194–201.
Weinshenker, B.G., Bulman, D., Carriere, W., Baskerville, J., Ebers, G.C., 1990. A comparison of sporadic and familial multiple sclerosis. Neurology 40 (9), 1354.
Werneck, L.C., Lorenzoni, P.J., Arndt, R.C., Kay, C.S.K., Scola, R.H., 2016. The immunogenetics of multiple sclerosis. The frequency of HLA-alleles class 1 and 2 is lower in southern Brazil than in the European population. Arq. Neuropsiquiatr. 74 (8), 607–616.