The immunogenetics of multiple sclerosis. The frequency of HLA-alleles class 1 and 2 is lower in Southern Brazil than in the European population

Imunogenética na esclerose múltipla. A frequência dos alelos HLA classe 1 e 2 no sul do Brasil são menores que da população Europeia

Lineu Cesar Werneck[1], Paulo José Lorenzoni[1], Raquel Cristina Arndt[1], Cláudia Suemi Kamoi Kay[1], Rosana Herminia Scola[1]

ABSTRACT

Objective: To study the HLA of class 1 and 2 in a multiple sclerosis (MS) population to verify the susceptibility for the disease in the Southern Brazil. **Methods:** We analyzed patients with MS and controls, by direct sequencing of the genes related to HLA DRB1, DQB1, DPB1, A, B and C alleles with high resolution techniques. **Results:** We found a lower frequency of all HLA alleles class 1 and 2 in MS and controls comparing to the European population. Several alleles had statistical correlation, but after Bonferroni correction, the only allele with significance was the HLA-DQB1*02:03, which has a positive association with MS. **Conclusions:** Our data have different frequency of HLA-alleles than the previous published papers in the Southeast Brazil and European population, possible due to several ethnic backgrounds.

Keywords: genetic predisposition to disease; HLA antigens; immunogenetics; major histocompatibility complex; multiple sclerosis.

RESUMO

Objetivo: Estudo do HLA classes 1 e 2 em pacientes com esclerose múltipla (EM) a fim de verificar a susceptibilidade para a doença em uma população do Sul do Brasil. **Métodos:** Foram analisados por sequenciamento direto de alta resolução os genes relacionados com os HLA DRB1, DQB1, DPB1, A, B e C em casos de EM comparados com uma população controle normal. **Resultados:** Foi encontrado uma frequência menor dos alelos dos HLA classe 1 e 2 nos casos de EM e controles quando comparado com a população Europeia. Diversos alelos mostraram correlação estatística, mas depois da correção de Bonferroni, somente o alelo do HLA-DQB1*02:03 foi positivo para a EM. **Conclusões:** Encontramos frequência diferente dos alelos do HLA relatados previamente nos Sudeste do Brasil e Europeus, possivelmente devido a origem étnica diferente da população estuda.

Palavras-chave: predisposição genética para doença; antígenos HLA; complexo principal de histocompatibilidade; esclerose múltipla.

The major histocompatibility complex (MHC), which is also known as the human leukocyte antigen system (HLA), is a set of specialized glycoproteins expressed on the surface of lymphocytes and encoded by genes located on chromosome 6p21.3. These glycoproteins are responsible for recognizing cells that contain a pathogenic organism, that are foreign to the body or whose surfaces have suffered changes[1.] Several polymorphisms have been found in the HLA genes, and this variability determines individual characteristics of the immune responses to infectious agents and foreign cells.

These responses are critical in cases of transplantation and in the pathogenesis of autoimmune diseases, such as ankylosing spondylitis, type 1 diabetes mellitus, narcolepsy, Reiter's syndrome, myasthenia gravis and multiple sclerosis (MS)[1,2,3]. The first link between MS and HLA was reported in 1973 and involved HLA-DR2. A similar association for other HLA class II has since been reported by other authors, who assumed that the disease is related to patient ethnicity and possible to environmental factors that are unknown yet[3,4]. A strong correlation was found between MS and HLA-DRB1*15:01 in

northern European countries[5]; DRB1*15:01, DRB1*03:01 and DRB1*04:05 in Italy (Sardinia Islands)[6]; DRB1*15:01-DQA1*01:02-DQB1*06:02, DRB1* 04:02-DQA1*03:01-DQB1*03:02 and DRB1*13:03-DQA1*05-DQB1*03:01 in Spain (in the province of Biscay)[7]; DRB1*15:01 and DQB1*0:602 in Ireland[8]; and DRB1*04:03 and DRB1*08:02 in Mexico[9]. An association was also found between MS and the DQA1*01:02 and DQB1*06:02 haplotypes in African-Brazilian patients whether the DRB1*15:01 allele was absent[10].

In recent years, researchers have turned their attention to HLA class I and its alleles. Initially performed with low resolution techniques, HLA typing showed that HLA-A*02 is negatively associated with the risk of MS (*i.e.*, it is associated with a protective effect/reduction in susceptibility) regardless of the presence of the DRB1*15 allele[11]. Haplotype studies have shown that, HLA-A*02 reduces susceptibility to the disease (protective effect) when associated with HLA-B*12 and HLA-Cw*05. The same occurred if this allele was associated with HLA-Cw*05 and HLA-B*44[11,12]. The presence of the allele Cw*05 in the haplotype reduce the expression of HLA-B12 and DRB1*15 and the susceptibility to the disease[11,13].

In light of the above, the aim of this study is to analyze the HLA class 1 and 2 profiles, with high resolutions technique, of MS patients of a Southern Brazil population to identify whether there is relationship between HLA type and susceptibility to MS in the Southern Brazilian population.

METHODS

Patients and controls

We analyzed 86 patients with a diagnosis of MS, 80 with the relapsing-remitting subtype and 6 with the primary progressive subtype, predominantly of white color, based on the 2010 McDonald criteria and 2013 revision[14]. The cases had followed up at the demyelinating disease outpatient clinic at the Hospital de Clínicas, Universidade Federal do Paraná. MS patients who fulfilled the following criteria were included: (1) patient born in the south of Brazil and lived there for most of his/her life; (2) patient's complete medical records available (both clinical and laboratory data that information on the clinical course of the disease was available); (3) patient evaluated with the Expanded Disability Status Scale (EDSS) during treatment or follow-up or data in the patient's records sufficient to allow the disability to be graded; and (4) findings of patient's MRI studies compatible with MS. We excluded MS patients who had incomplete medical records, conflicting data, clinically isolated syndrome, neuromyelitis optica or incomplete outcome data that did not allow other diseases to be ruled out (Table 1).

The control group consisted of bone marrow donors who fulfilled the following criteria: 1) absence of any disease; 2) over 30 years old; 3) born and had always lived in southern Brazil; 4) only two unrelated members of the transplant recipient's household could be included (mother and father), and in the parents' absence only one sibling could be included; and 5) HLA screening performed in Curitiba at the Immunogenetics Laboratory, Hospital de Clínicas, Universidade Federal do Paraná (All data were kindly supplied by Dr. Noemi Farah Ferreira and her laboratory team). The number of controls included in the study was 606 donors for HLA-DRB1 (1212 alleles), 220 for HLA-DQB1 (440 alleles), 133 for HLA-DPB1 (266 alleles), 127 for HLA-A (254 alleles), 168 for HLA-B (336 alleles) and 111 for HLA-C (222 alleles).

The study was approved by the Ethics Committee for Research with Humans at the Hospital de Clínicas, Universidade Federal do Paraná. All patients agreed to participate and signed a consent form.

HLA Typing

Genomic DNA was extracted from blood using a standard phenol-chloroform technique after treatment with proteinase-K and then amplified by polymerase chain reaction (PCR) to obtain gene fragments (exons) related to HLA class I and II using oligonucleotides flanking specific regions of the following exons: HLA-A (exons 2, 3 and 4), HLA-B (exons 2, 3 and 4), HLA-C (exons 2, 3 and 4), HLA-DRB1 (exon 2), HLA-DPB1 (exon 2) and HLA-DQB1 (exons 2 and 3). PCR was performed separately for each exon of these different genes using a conventional method with *Taq* DNA polymerase (Abbott Molecular Diagnostics) and a SBT (sequence based typing) kit (AlleleSEQR-SBT) (Atria Genetics) following the manufacturers' instructions. PCR products were purified by ExoSAP-IT® (USB, Cleveland, Ohio). The purified

Table 1. Characteristics of multiple sclerosis (MS) patients and control group.

Variable	MS (n = 86)	Controls (n = 606)
Age	40.87 ± 11.65 (18−63)	49.53 ±13.11 (30−88)
Age onset of symptoms	29.95 ± 9.81 (12−50)	-
Disease duration	10.91 ± 6.87 (1−34)	-
Initial EDSS score	2.43 ±1.69 (0−7.5)	-
Follow-up EDSS score	3.36 ± 2.47 (0−8.5)	-
Gender		
Females	60 (69.8%)	304 (50.16%)
Males	26 (30.2%)	302 (49.84%)
Color skin		
White	83 (96.6%)	578 (95.6%)
Brown (mulatto)	3 (3.4%)	11 (1.8%)
Black	0	9 (1.4%)
Yellow (Asian)	0	5 (0.8%)
Amerindian	0	2 (0.4%)

EDSS: expanded disability status scale.

PCR products were then subjected to a second round of PCR using Big Dye Mix® (Applied Biosystems) followed by purification of the products by the isopropanol method. The amplified fragments were directly sequenced in forward and reverse directions by fluorescent capillary electrophoresis using POP-6 polymer (Applied Biosystems, Foster City, CA) in an ABI PRISM 3100 and 3130 Avant Genetic Analyzers® (Hitachi High-Technologies Corporation, Tokyo, Japan). The HLA sequences were compared with reference sequences by high-resolution HLA typing using Assign SBT software (Conexio-Genomics, Fremantle, Australia) and uTYPE®HLA Analysis Software (Thermo Fisher Scientific, Waltham, MA).

Statistical analysis

Descriptive statistics were calculated for age (MS cases and controls) and age at onset of symptoms, EDSS score at disease onset and EDSS score at the last evaluation (MS cases). The relationships between the MS cases and controls were calculated with Pearson's chi-square test using Yates' correction. Statistical significance was considered for p < 0.05. These values were then corrected by Bonferroni methods according the number of alleles analyzed. The Odds Ratio was estimate with the Mantel-Haenszel statistic method.

RESULTS

The HLA-DRB1 was sequenced in 86 cases, HLA-DQB1 and DPB1 in 85, HLA-A in 64, HLA-B in 65 and HLA-C in 85. These data were not uniform because of ambiguities and technical artifacts found in some patients.

HLA-DRB1: We found 52 different alleles in MS and 44 in controls. Of those, 19 alleles occurred only in MS and 11 only in controls. The most common allele in MS patients and controls was DRB1*15:01, which was present in a higher percentage of individuals in the MS group. This was followed by *03:01 and *07:01 alleles. DRB1*15:01, *11:03 and *04:06 alleles. They were statistically significant to MS, but all of them were non significant after Bonferroni correction for the 63 alleles. The same occurred with DRB1*11:01 and *10:01 alleles in the controls (Table 2).

HLA-DQB1: We found 35 different alleles in MS and 17 in controls. Of those, 19 alleles occurred only in MS and only 1 control did not have the same allele that MS. The most common allele was DQB1*06:02 in MS, followed by *02:01 and *03:02. The DQB1*02:03, *03:69 and *04:03 alleles were statistically significant, whereas in the controls DQB1 *03:01 and *02:02 alleles were more frequent. After the Bonferroni

Table 2. Frequency of the HLA-DRB1 in multiple sclerosis and control group.

Alleles	MS	%	Controls	%	p	OR (95%CI)	p^c
DRB1	86 Cases		606 Cases				
*15:01	24	14.0	98	8.1	0.017	1.843 (1.14–2.97)	NS
*03:01	17	9.9	85	7.0	0.233	1.454 (0.84–2.52)	NS
*07:01	16	9.3	180	14.9	0.066	0.588 (034–1.00)	NS
*13:01	11	6.4	71	5.9	0.643	0.784 (0.37–1.65)	NS
*13:02	8	4.7	38	3.1	0.418	1.507 (0.69–3.28)	NS
*11:04	7	4.1	32	2.6	0.416	1.564 (0.68–3.60)	NS
*01:01	6	3.5	52	4.3	0.164	0.396 (0.12–1.28)	NS
*04:04	6	3.5	33	2.7	0.748	1.291 (0.53–3.13)	NS
*01:02	4	2.3	22	1.8	0.872	1.288 (0.43–3.78)	NS
*08:01	4	2.3	32	2.6	1.000	0.878 (0.31–2.51)	NS
*08:07	4	2;3	8	0.7	0.078	3.583 (1.07–12.03)	NS
*11:01	4	2.3	79	6.5	0.046	0.341 (0.12–0.94)	NS
*16:01	4	2.3	29	2.4	1.000	0.971 (0.34–2.80)	NS
*04:02	3	1.7	30	2.5	0.748	0.699 (0.21–2.31)	NS
*11:03	3	1.7	3	0.2	0.030	7.154 (1.43–35.73)	NS
*14:01	3	1.7	63	5.2	0.072	0.324 (0.10–1.04)	NS
*04:06	2	1.2	0	0	0.007	0	NS
*10:01	0	0	44	3.6	0.021	0	NS
Others alleles	35 Cases	-	27 Cases	-	-	-	NS
	46	26.8	313	25.9			
Total	172	100	1212	100	-	-	-

MS: multiple sclerosis; p: probability; OR: odds ratio; CI: confidence interval; p^c: probability after Bonferroni correction for 53 multiple comparisons for p < 0.05; NS: non significant.

correction for 36 alleles, only the allele DQB1*02:03 was significant (p = 0.0027) (Table 3).

HLA-DPB1: We found 30 different alleles in MS and 22 in controls. In MS, 14 alleles occurred only in this group and 6 controls did not have similar MS allele. The most common alleles in the MS group and controls were *04:01, *02:01 and *04:02. The *23:01 and *51:01 alleles were statistically significant, but they lost the significance after Bonferroni correction for the 36 alleles (Table 4).

HLA-A: We found 42 different alleles in MS and 32 in controls. In MS, 22 alleles occurred only in this group and 12 controls did not have similar allele in MS. The most common alleles in the MS group were *03:01 and *24:02. The alleles *24:02 and *02:01 had statistical significance, but they become non significant after Bonferroni correction for the 57 alleles (Table 5).

HLA-B: We found 60 different alleles in MS and 76 in controls. In MS group, 25 alleles did not occurred in the controls and 42 of the controls did not have similar allele in MS group. The most common allele in the MS group and controls were *07:02, *51:01 and *35:01. There is a statistical relation with alleles *35:01, *44:02, *14:06, and *52:01, but all of them lost the statistical significance after Bonferroni correction for the 97 alleles (Table 6).

HLA-C: We found 39 different alleles in MS and 30 in controls. In MS group, 20 alleles did not occurred in controls and

11 of the controls did not have the same MS allele. The most common alleles in the MS group and controls were *04:01, *07:02 and *08:02. HLA-C*07:27 was the only allele with statistical correlation, but it lost the significance after Bonferroni correction (Table 7).

DISCUSSION

HLA-DRB1*15:01 is considered a major susceptibility allele, but other HLA alleles in the haplotype, as well as the way that they were transmitted, can increase or decrease this susceptibility. These other alleles (of the same or different class) can determine epistatic interactions and modifications or epigenetic variations that may change the expression of nearby genes, determining greater or lesser susceptibility to MS[13,15,16].

Although the frequency of DRB1*15:01 in our study was increased, the frequencies of this and other alleles were lower for both, MS patients and controls, compared with that reported in Europeans[17]. We found association but after the Bonferroni correction disappeared. This difference may be explained by the number of cases studied and the influence of the mixing of European, African and indigenous genes in Brazil[18] (Figure 1).

In three Brazilian studies the percentage of the DRB1*15:01 allele was high, especially in white individuals[19,20] and women[21].

Table 3. Frequency of the HLA-DQB1 in multiple sclerosis and control group.

Allele	MS	%	Controls	%	p	OR (95% CI)	pᶜ
DQB1	85 cases		220 cases				
*06:02	29	17.1	55	12.5	0.182	1.440 (0.88–2.35)	NS
*02:01	18	10.6	42	9.5	0.813	1.122 (0.62–2.01)	NS
*03:02	16	9.4	40	9.1	1.000	1.039 (0.56–1.91)	NS
*03:01	15	8.8	84	19.1	0.003	0.410 (0.30–0.79)	NS
*05:01	13	7.6	53	12.0	0.155	0.605 (0.32–1.14)	NS
*04:02	12	7.1	21	4.8	0.358	1.515 (0.73–3.15)	NS
*06:03	9	5.3	27	6.1	0.838	0.855 (0.39–1.86)	NS
*02:02	6	3.5	49	11.1	0.005	0.292 (0.12–0.69)	NS
*02:03	6	3.5	0	0	0.000	0	0.0027
*05:03	5	2.9	13	3.0	1.000	0.995 (0.50–2.84)	NS
*05:02	4	2.4	10	2.3	1.000	1.036 (0.32–3.35)	NS
*06:08	4	2;4	0	0	0.008	0	NS
*03:69	3	1.8	0	0	0.032	0-	NS
*04:03	3	1.8	0	0	0.032	0-	NS
*06:09	2	1.2	3	0.7	0.915	1.734 (0.29–10.47)	NS
*06:11	2	1.2	0	0	0.136	0	NS
Other alelles	20 cases	-	6 Cases	-	-	-	NS
	23	13.4	43	9.8			
Total	170	100%	440	100%	-	-	-

MS: multiple sclerosis; p: probability; OR: odds ratio; CI: confidence interval; pᶜ: probability after Bonferroni correction for 36 multiple comparisons for p < 0.05; NS: non significant.

Table 4. Frequency of the HLA-DPB1 in multiple sclerosis and control group.

Allele DPB1	MS 85 Cases	%	Controls 133 Cases	%	p	OR (95%CI)	pᶜ
*04:01	44	25.9	68	25.6	1.000	0.017 (0.65–1.58)	NS
*02:01	18	10.6	37	13.9	0.384	0.733 (0.40–1.33)	NS
*04:02	18	10.6	40	15.0	0.234	0.669 (0.37–1.21)	NS
*03:01	12	7.1	32	12.0	0.129	0.55 (0.28–1.11)	NS
*01:01	10	5.9	14	5.3	0.951	1.125 (0.49–2.59)	NS
*05:01	9	5.3	10	3.8	0.600	1.431 (0.57–3.60)	NS
*10:01	9	5.3	9	3.4	0.465	1.596 (0.62–4.106)	NS
*23:01	9	5.3	1	0.4	0.003	14.814 (1.90–118.01)	NS
*14:01	5	2.9	12	4.5	0.567	0.641 (0.22–1.85)	NS
*13:01	4	2.4	6	2.3	1.000	1.044 (0.29–3.76)	NS
*51:01	4	2.4	0	0	0.046	0	NS
*71:01	3	1.8	0	0	0.114	0	NS
*06:01	2	1.2	4	1.5	1.000	0.780 (0.14–4.30)	NS
*11:01	2	1.2	10	3.8	0.191	0.305 (0.07–1.41)	NS
*20:01	2	1.2	0	0	0.295	0	NS
*57:01	2	1.2	0	0	0.295	0	NS
Other alelles	14 Cases	-	10 Cases	-	-	-	-
	17	9.7	27	8.5			
Total	170	100	266	100	-	-	-

MS: multiple sclerosis; p: probability; OR: odds ratio; CI: confidence interval; pᶜ: probability after Bonferroni correction for 36 multiple comparisons for p < 0.05; NS: non significant.

Table 5. Frequency of the in multiple sclerosis and control group.

Allele HLA-A	MS 62 cases	%	Controls 195 cases	%	p	OR (95% CI)	pᶜ
03:01	20	16.1	37	9.5	0.040	1.835 (1.021–3.297)	NS
24:02:00	17	13.7	39	10.0	0.322	1.430 (0.777–2.630)	NS
02:01	11	8.9	79	20.3	0.004	0.383 (0.197–0.746)	NS
01:01	11	8.9	42	10.8	0.663	0.807 (0.402–1.619)	NS
23:01	7	5.6	19	4.9	0.915	1.168 (0.479–2.848)	NS
68:01:00	5	4.0	10	2.6	0.589	1.597 (0.535–4.763)	NS
11:01	5	4.0	16	4.1	1.000	0.982 (0.352–2.738)	NS
68:02:00	4	3.2	4	1.0	0.191	3.217 (0.792–13.057)	NS
31:01:00	3	2.4	21	5.4	0.270	0.439 (0.129–1.499)	NS
25:01:00	3	2.4	6	1.5	0.796	1.587 (0.391–6.440)	NS
32:01:00	2	1.6	13	3.3	0.493	0.475 (0.106–2.136)	NS
26:01:00	2	1.6	11	2.8	0.676	0.565 (0.123–2.584)	NS
33:01:00	2	1.6	6	1.5	1.000	1.049 (0.209–5.266)	NS
29:02:00	1	0.8	15	3.8	0.161	0.203 (0.027–1.554)	NS
30:01:00	0	0	11	2.8	0.059	0	NS
03:02	0	0	8	2.1	0.234	0	NS
Other alleles	28 cases	-	21 cases	-	-	-	NS
	31	25.2	53	13.6			
Total	124	100	390	100	-	-	-

MS: multiple sclerosis; p: probability; OR: odds ratio; CI: confidence interval; pᶜ: probability after Bonferroni correction for 57 multiple comparisons for p < 0.05. NS: non significant.

Table 6. Frequency of HLA-B in multiple sclerosis and control group.

Allele HLA B	MS 69 cases	%	Controls 234 cases	%	p	OR (95%CI)	p^c
07:02	15	10.9	31	6,6	0.098	1.719 (0.899–3.287)	NS
35:01:00	10	7.2	11	2,4	0.012	3.246 (1.348–7.814)	NS
51:01:00	10	7.2	16	3,4	0.051	2.207 (0.978–4.982)	NS
14:02	7	5.1	21	4.5	0.773	1.137 (0.473–2.735)	NS
18:01	7	5.1	11	2.4	0.171	2.220 (0.844–5.841)	NS
44:02:00	7	5.1	7	1.5	0.033	3.519 (1.213–10.213)	NS
08:01	5	3.6	19	4.1	1.000	0.888 (0.326–2.424)	NS
14:01	5	3.6	8	1.7	0.303	2.162 (0.696–6.718)	NS
15:01	5	3.6	13	2.8	0.819	1.316 (0.461–3.758)	NS
14:06	3	2.2	0	0	0.012	0	NS
40:01:00	3	2.2	4	0.9	0.411	2.578 (0.570–11.659)	NS
41:01:00	3	2.2	1	0.2	0.057	10.378 (1.071–100.577)	NS
49:01:00	3	2.2	10	2.1	1.000	1.108 (0.276–3.751)	NS
52:01:00	1	0.7	24	5.1	0.041	0.135 (0.018–1.007)	NS
15:03	1	0.7	15	3.2	0.195	0.220 (0.029–1.684)	NS
38:01:00	0	0	10	2.1	0.177	0	NS
Other alleles	45 cases		59 cases	-	-	-	NS
	53	38.4	267	55.0			
Total	138	100	468	100	-	-	-

MS: multiple sclerosis; p: probability; OR: odds ratio; CI: confidence interval; p^c: probability after Bonferroni correction for 54 multiple comparisons for p < 0.05; NS: non significant.

Table 7. Frequency of the HLA-C in multiple sclerosis and control group.

Allele HLA-C	MS 85 Cases	%	Controls 111 Cases	%	p	OR (95%CI)	p^c
*04:01	26	15.3	32	14.4	0.921	1.072 (0.61–1.88)	NS
*07:02	24	14.1	31	14.0	1.000	1.013 (0.57–1.80)	NS
*08:02	13	7.6	9	4.1	0.190	1.960 (0.82–4.70)	NS
*05:01	11	6.5	11	5.0	0.671	1.327 (0.56–3.14)	NS
*07:01	10	5.9	21	9.5	0.266	0.598 (0.27–1.31)	NS
*03:04	9	5.3	13	5.9	0.986	0.899 (0.37–2.15)	NS
*06:02	9	5.3	25	11.3	0.58	0.440 (0.20–0.97)	NS
*01:02	7	4.1	4	1.8	0.286	2.340 (0.67–8.13)	NS
*03:03	7	4.1	9	4.1	1.000	1.016 (0.37–2.79)	NS
*12:03	6	3.5	13	5.9	0.409	0.588 (0.22–1.58)	NS
*07:27	5	2.9	0	0	0.034	0	NS
*15:02	5	2.9	2	0.9	0.260	3.333 (0.64–17.39)	NS
*17:01	5	2.9	3	1.4	0.458	2.212 (0.52–9.39)	NS
*02:02	4	2.4	10	4.5	0.388	0.511 (0.16–1.66)	NS
*04:09	2	1.2	0	0	0.365	0	NS
*14:02	2	1.2	0	0	0.365	0	NS
*15:05	2	1.2	3	1.4	1.000	0.869 (0.14–5.26)	NS
Other	22 cases	-	16 cases	-	-	-	NS
	23	13.6	36	15.8			
Total	170	100	222	100	-	-	-

MS: multiple sclerosis; p: probability; OR: ODDS RATIO; CI: confidence interval; p^c: probability after Bonferroni correction for 54 multiple comparisons for p < 0.05; NS: non significant.

However, they mixed high-resolution with low-resolution techniques, as well as they have a low number of controls population to identify the DRB1*15:01 allele.

Although DQB1*06:02 was the most frequent allele, we failed to find an association between this allele and MS, as reported in
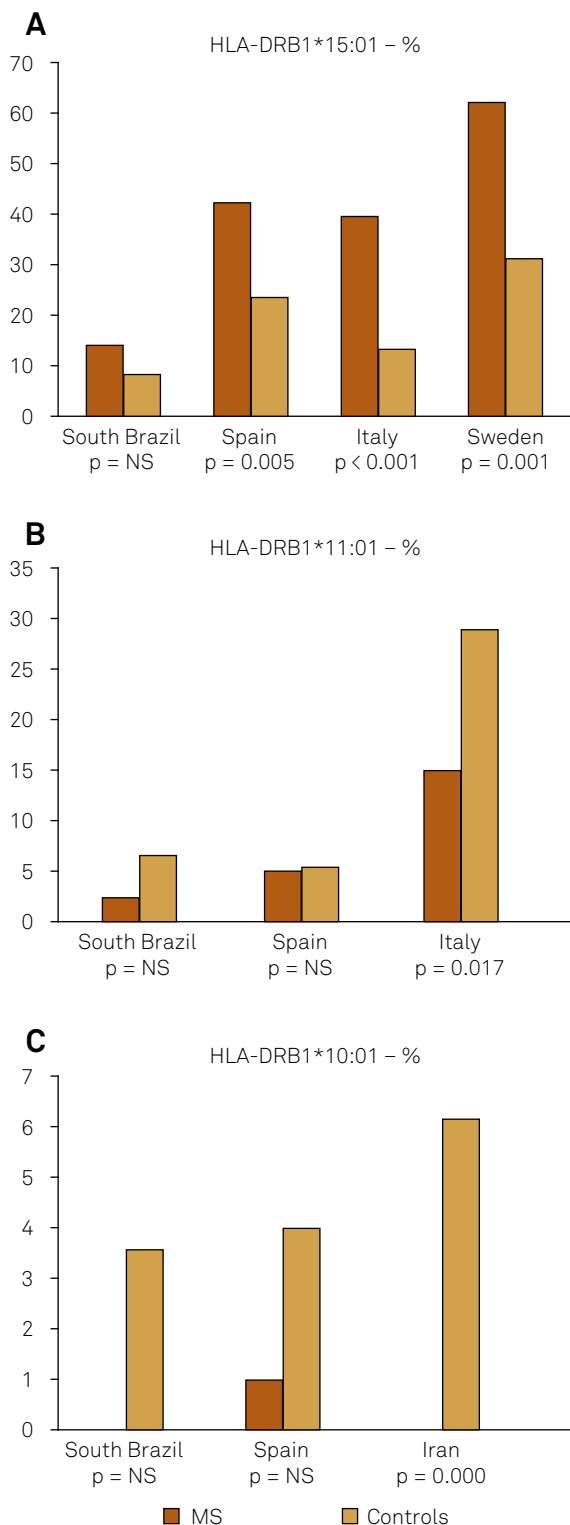
HLA-DRB1*15:01 – %

South Brazil
p = NS
Spain
p = 0.005
Italy
p < 0.001
Sweden
p = 0.001

HLA-DRB1*11:01 – %

South Brazil
p = NS
Spain
p = NS
Italy
p = 0.017

HLA-DRB1*10:01 – %

South Brazil
p = NS
Spain
p = NS
Iran
p = 0.000

■ MS □ Controls

Figure 1. (A) Frequency of HLA-DRB1*15:01 alleles in South Brazil, Spain[7], Italy[6] and Sweden[5]; (B) Frequency of HLA DRB1*11:01 alleles in South Brazil, Spain[7] and Italy[16]; (C) Frequency of HLA-DRB1*10:01 alleles in South Brazil, Spain[7] and Iran[23].

Spain (Basque Country)[7], Ireland[8], Italy (Sardinia)[22], Iran[23], China (Hong Kong)[24], Colombia[25] and Afro-Brazilian (Rio de Janeiro)[10]. The only DQB1 allele which remained with statistical significance after Bonferroni correction was the *02:03. This association was not previously reported.

The allele DQB1*03:01 was associated with MS in Spain[7] but have a reduced susceptibility in Iran[23]. However, previous studies showed that the risk of MS increases by 50% in the presence of HLA-DPB1*03:01[26] (Figure 2).

In the analysis of HLA-DPB1, we did not find the *03:01 allele associated with MS, as reported in Italy (Sardinia)[6] and several European countries[26]. Although this association was only found in the absence of DQB1*06:02 allele in Australia[27]. The DPB1*05:01 allele showed no association with MS in our series, in contrast with the findings for other series from South China[28] (Figure 3).

In our series, before Bonferroni correction, there was a positive association between MS and HLA-A*03:01 (increased susceptibility) and a negative association for HLA-A*02:01 (increased in controls, protective effect). The reduced susceptibility to MS (protective effect) of HLA-A*02:01, *68:01, *02:05 and *06:06 alleles have been reported[26]. It has been shown that if HLA-A*03:01 allele is associated with HLA-B*07:02 allele, the risk of MS increases, but if it is associated with HLA-A*02:01 allele, the risk is reduced[5] (Figure 4).

HLA-B*35:01, *44:02, and *14:06 alleles were correlated with disease susceptibility in our series, but after Bonferroni correction no allele had significance. It is reported that the HLA-B*44 allele when present reduce susceptibility to MS, slowing disease progression and preserving brain volume in North American patients [15,29]. However, in our series, it was more frequent in MS cases, than the previous reports, which have more cases in the control groups (Figure 5).

HLA-C *07:27 allele was found only in the MS group, suggesting an association with susceptibility to the disease. In addition, our results failed to reveal an association between HLA-C*05, which was shown to have a protective effect in some MS population[29]. However, it is difficult to make any correlation of the HLA-C because most of the previous studies are in low resolution.

Our HLA results probably could be explained by two main hypothesis: first, the different distribution of the ethnic background into Brazil; and second, the different resolutions techniques used in the previous studies.

Regarding the ethnic Brazilian background, the differences found comparing with Europeans could be due to the variation in the Brazilian population because of the many immigrants with different ethnic backgrounds who arrived in Brazil and gradually mixed with the local inhabitants over the last 500 years[30].

Brazil was colonized mainly by the Portuguese, who mixed with native Brazilian Indians in the first centuries following colonization. Later, African slaves were introduced to

the country, mainly to the Southeast and Northeast to work on sugarcane plantations. In the mid 19th century European immigrants began to arrive in Brazil. However, they did not settle evenly throughout the country and tended to concentrate in certain areas according to their ethnic descent[30].

The Southeast, for example, had large numbers of Portuguese and Spanish people from Galicia, as well as Arabs, Jews and
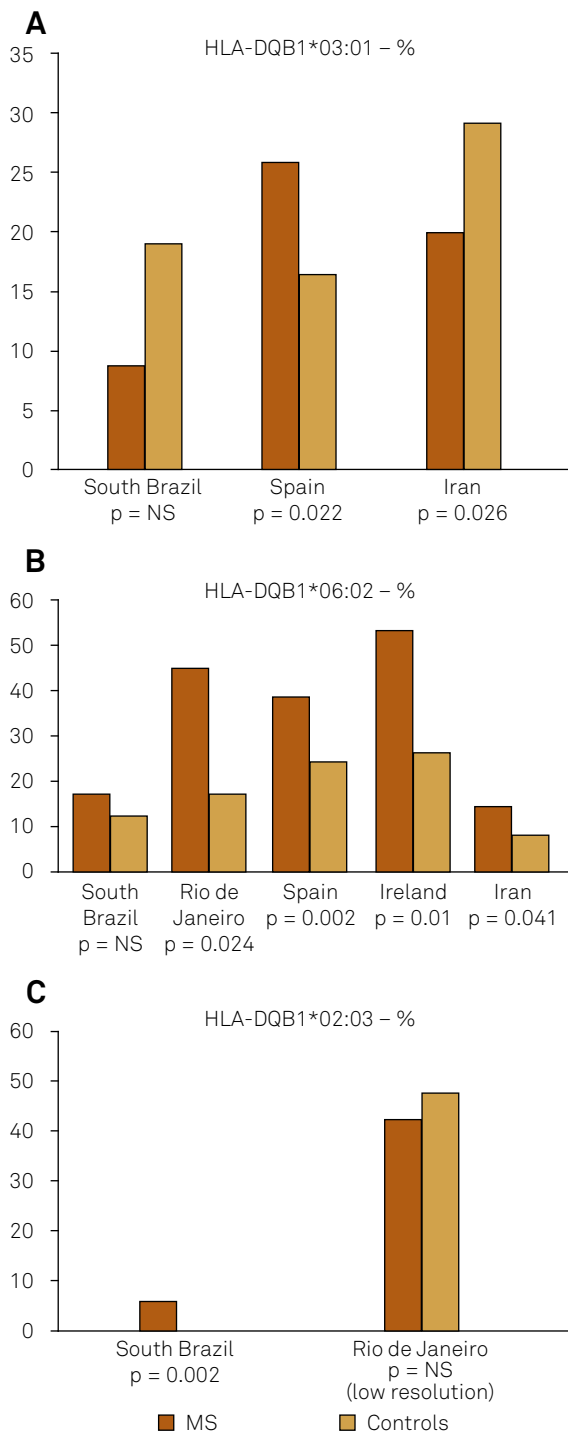
A HLA-DQB1*03:01 – %

B HLA-DQB1*06:02 – %

C HLA-DQB1*02:03 – %

Figure 2. (A) Frequency of HLA-DQB1*03:01 allele in South Brazil, Spain[7] and Iran[23]; (B) Frequency of HLA-DQB1*06:02 allele in South Brazil, Rio de Janeiro (Afro-Brazilian)[10], Spain[7], Ireland[8] and Iran[23]; (C) Frequency of HLA-DQB1*02:03 allele in South Brazil and Rio de Janeiro (Afro-Brazilian)[19].
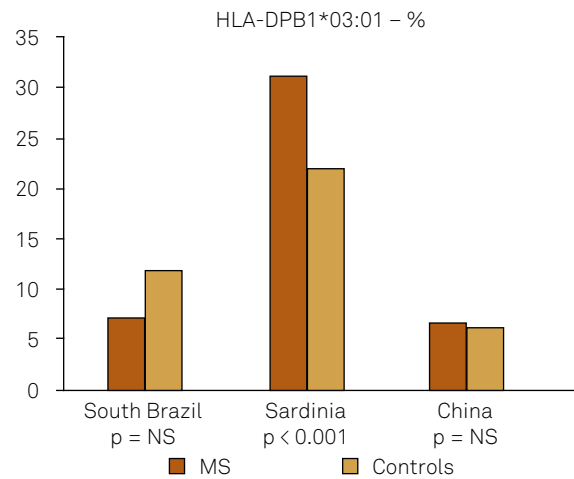
HLA-DPB1*03:01 – %

Figure 3. Frequency of HLA-DPB1*03:01 allele in South Brazil, Sardinia (Italy)[6] and China[28].
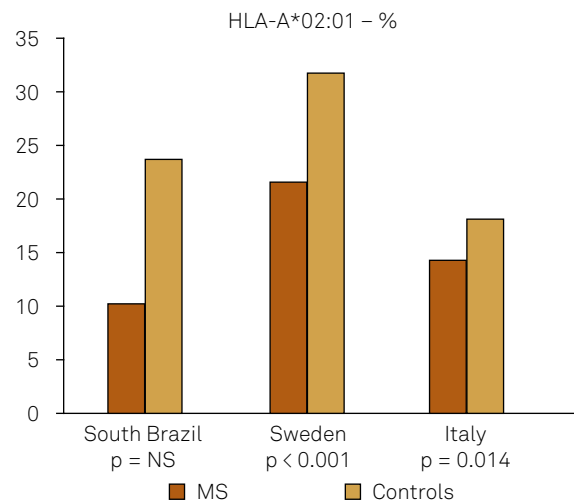
HLA-A*02:01 – %

Figure 4. Frequency of HLA-A*02:01 allele in South Brazil, Sweden[5] and Italy[16].
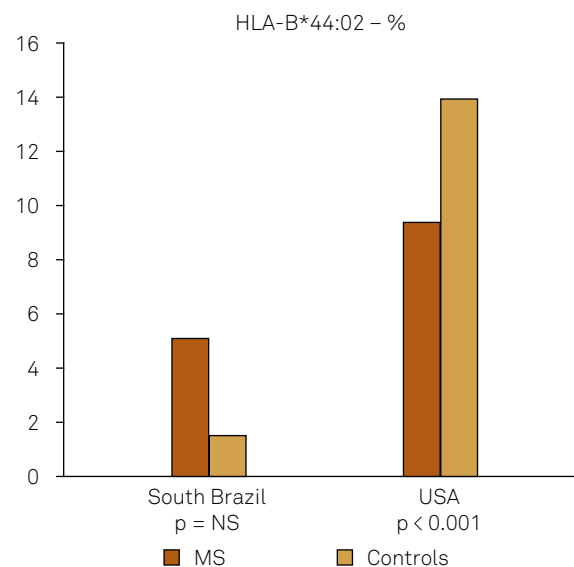
HLA-B*44:02 – %

Figure 5. Frequency of HLA-B*44:02 allele in South Brazil and United States[17].

Japanese in smaller numbers. The Italians settled in the Southeast and South and constituted one of the largest immigrant population in Brazil, while the Germans, Polish and Ukrainians settled mainly in the South[30].

Genetic studies have shown that the white Brazilian population has genes of Europeans, African and indigenous origin. The distribution of these European genes is higher in the South (where our study was done), there is a greater proportion of white individuals and a smaller proportion of black individuals in this area[31].

Regarding the different resolutions techniques used in the previous studies, the admixture of the population of several ethnic origins increased the number of different alleles which are easier to analyses with techniques of high resolution. At present time, if the typing the HLA was performed only with high resolutions techniques, the number of alleles in each class increased too much (http://hla.alleles.org/alleles/class1.html; http://hla.alleles.org/alleles/class2.html). Therefore, if the Bonferroni correction was applied in this situation (for the total number of alleles), most of the studies lost their statistical significance.

In some of the previous HLA studies performed in Brazil, the analysis grouped together techniques of low and high resolution, which rise difficulties to compare with our results that were performed only with high resolution techniques[10,19,20].

We believe that the mainly factor to our different results was the different ethnic backgrounds into Brazil which can really results in many possible genetic combinations in the Brazilian population. Furthermore, beside the type of HLA and ethnic diversity, probably climate with less sunlight, different environmental conditions and dietary habits could play a role in the triggering and incidence of MS in southern Brazil different from other countries[8,32,33,34,35].

In conclusion, our data have different frequency of HLA alleles in Southern Brazilian population than the previous published with the Southeast Brazil region and Europeans. We also speculate that further studies of HLA regarding the MS susceptibility in the European Countries in the next century may find different results from today when using high resolution techniques and large control groups. We do not know the effect of the dilution of the original HLAs alleles due to the massive immigration which is occurring in the present days, similar that occurred with the original Brazilians inhabitants over the last 500 years.

Acknowledgements

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