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ARTICLE

HLA-alleles class I and II associated with genetic susceptibility to neuromyelitis optica in Brazilian patients

Alelos HLA classes I e II associados à suscetibilidade genética a neuromielite óptica em pacientes brasileiros

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ABSTRACT

Objective: To study the genetic susceptibility to neuromyelitis optica (NMO) as well as the relationship between HLA genotypes and susceptibility to the disease in the southern Brazilian population. **Methods:** We analyzed patients with NMO, who met criteria for Wingerchuk's diagnosis of NMO, with detected serum anti-AQP4-IgG antibody. The HLA genotyping was performed by high-resolution techniques (Sanger sequencing) in patients and controls. The HLA genotypes were statistically compared with a paired control population. **Results:** The HLA genotyping revealed the diversity of the southern Brazilian population whose HLA profile resembled European and Asian populations. Some alleles had statistical correlations with a positive association (increased susceptibility) with NMO, particularly the *HLA-DRB1*04:05* and **16:02*. **Conclusions:** In our study, the HLA genotype was different to that previously reported for other Brazilian populations. Although our study had a small cohort, HLA genotypes were associated with increased susceptibility to NMO for *HLA-DRB1*04:05* and **16:02*. The alleles of HLA class I *HLA-A*02:08* and **30:09*, *HLA-B*08:04* and **35:04* showed an association before the Bonferroni correction.

Keywords: Genetic predisposition to disease; HLA antigens; immunogenetics; major histocompatibility complex; neuromyelitis optica.

RESUMO

Objetivo: Estudar a suscetibilidade genética a neuromielite óptica (NMO) assim como sua relação com o genótipo HLA na população do sul do Brasil. **Métodos:** Nós analisamos pacientes com NMO que preenchiam os critérios diagnósticos de Wingerchuk para NMO, com presença do anticorpo anti-AQP4-IgG no soro. O genótipo HLA foi realizado usando técnicas de alta resolução (sequenciamento de Sanger) em pacientes e controles. Genótipos HLA foram estatisticamente comparados com uma população controle pareada. **Resultados:** Genotipagem HLA revelou a diversidade da população sul brasileira cujo perfil HLA lembra as populações europeia e asiática. Alguns alelos tiveram correlação estatística com associação positiva (suscetibilidade aumentada) com NMO, particularmente o *HLA-DRB1*04:05* e **16:02*. **Conclusões:** Em nosso estudo, o genótipo HLA foi diferente do previamente relatado em outras populações brasileiras. Embora o número de pacientes tenha sido pequeno, HLA específicos foram associados com suscetibilidade aumentada a NMO para *HLA-DRB1*04:05*, **16:02*. Os alelos HLA classe I *HLA*02:08* e *30:09, *HLA-B*08:04* e **35:04* tiveram associação antes da correção de Bonferroni.

Palavras-chave: Predisposição genética para doença; antígenos HLA; imunogenética; complexo principal de histocompatibilidade; neuromielite óptica.

Neuromyelitis optica (NMO) is an inflammatory disease of the central nervous system that has been considered a "channelopathy", primarily following the description of a specific IgG antibody against the aquaporin-4 channel (AQP4)[1]. In addition,

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the term "astrocytopathy" has been proposed because histopathological studies have shown that astrocyte injury is the primary event, which occurs in the active lesions of NMO[2,3].

The major histocompatibility complexes are very dense and contain a polymorphic genomic region that expresses the human leukocyte antigen (HLA) present in the membrane of the T-cell receptors. Their high polymorphisms are due to variation in the extracellular domains forming the walls of the cleft where the peptides bind[4]. The HLA class II alleles are more frequently studied, but class I alleles are involved in the presentation of endogenous antigens and in the inhibition of natural killer cells, which could play a role in autoimmune diseases[5].

Although the HLA system has been studied in multiple sclerosis since the early 1970s, few studies have described the HLA system in NMO[6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22]. Several studies have associated specific HLA class II alleles with susceptibility to NMO[8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23]. Recent studies have shown a relationship between NMO and HLA class II, particularly for the *DPB1*05:01*, *DRB1*03* and *DRB1*16:02* alleles[7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22]. However, HLA class I alleles have only rarely been studied in NMO[10].

In Brazilian NMO patients, to the best of our knowledge, only three studies in Brazil have analyzed HLA class II alleles, and HLA class I alleles have not yet been studied[10,11,12,21]. In addition, no study has been done in NMO patients from a southern Brazilian population that has a large proportion of individuals descended from Europeans.

In this study, we analyzed HLA class I and II profiles, with genotyping by high resolution techniques, among patients with NMO, born and raised in southern Brazil, to identify whether there is a relationship between the HLA genotype and susceptibility to NMO in the southern Brazilian population.

METHODS

Patients

We analyzed 768 patients with demyelinating disorders admitted from 2000 to 2018 at the outpatient clinic of the Hospital de Clínicas of Universidade Federal do Paraná (Curitiba, Brazil). Neuromyelitis optica was identified according Wingerchuk's diagnostic criteria[24,25,26,27]. Neuromyelitis optica was defined by positive anti-AQP4-IgG antibody serum status and the presence of at least one of the following: 1) single, recurrent or simultaneous bilateral optic neuritis; 2) longitudinally extensive transverse myelitis (≥ 3 vertebral segments); or 3) recurrent brainstem, hypothalamic or cerebral symptoms[24,25,26]. We found 23 patients with NMO. We excluded five patients with negative AQP4-IgG serum status and three patients who were not born in the southern region of Brazil. Thus, we included 15 patients who fulfilled the criteria for NMO with positive AQP4-IgG serum status. We classified patients based on skin color. Female gender and white skin color were predominant in our sample. The age of onset had a median of 37 (range 24–63) years, the Expanded Disability Status Scale median was 6.0 (range 2.5–8.5) (Table 1). The indirect immunofluorescence technique was used for AQP4-IgG detection in all patient samples. Other serum antibodies were detected in 13 patients (Table 1). Other concomitant diseases included autoimmune disease in four patients and two with neoplasm (breast or lung) after the diagnosis of NMO (Table 1). Brain magnetic

Table 1. Clinical, laboratory and radiological findings of NMO patients from southern Brazil.

Variable	Number (%)
Gender	
Female	14 (93.3%)
Male	1 (6.7%)
Skin color	
White	12 (80.0%)
Brown (mulatto)	3 (20.0%)
Age at onset (median in years)	37 (range 24-63)
EDSS score (median)	6.0 (2.5-8.5)
Clinical presentation	
Optic neuritis and myelitis	13 (86.6%)
Optic neuritis	1 (6.7%)
Longitudinal extensive myelitis transverse	1 (6.7%)
Abnormal laboratory findings	
Anti-AQP4-IgG antibody	15 (100%)
Antinuclear antibody	6 (40.0%)
Thyroid peroxidase antibody	2 (13.3%)
Thyroglobulin antibody	3 (20.0%)
Antiphospholipid antibody	4 (26.6%)
Antimitochondrial antibody	1 (6.7%)
Antiendomysial antibody	1 (6.7%)
Associated autoimmune Disorders	
Hypothyroidism	3 (20.0%)
Antiphospholipid syndrome	1 (6.7%)
Associated cancer	
Breast cancer	1 (6.7%)
Bronchoalveolar (lung) cancer	1 (6.7%)
Brain MRI findings	
Normal	9 (60%)
Abnormal unspecific abnormality	4 (26.6%)
"multiple sclerosis" radiological criteria	2 (13.3%)
Cervical MRI findings	
Longitudinally extensive myelitis	14 (93.3%)
Normal	1 (6.7%)

HLA: human leukocyte antigen; NMO: neuromyelitis optica; MRI: magnetic resonance image; AQP4-IgG: antiaquaporin-4 immunoglobulin; EDSS: Expanded Disability Status Scale

resonance imaging was normal in nine patients and abnormal in six patients, and cervical magnetic resonance imaging showed longitudinally extensive transverse myelitis in all patients except one (Table 1).

Controls

The control group comprised 606 healthy donors of bone marrow transplant from our hospital. Only individuals who were born and raised in the southern regions of Brazil, mostly in the Paraná State, were selected. All were over 30 years old and were unrelated. The number of individuals in each division of the control group was calculated separately for each HLA gene, based on the number of alleles studied in the disease groups. These control groups were also statistically paired by skin color. The control groups had 384 alleles of *HLA-A*, 462 alleles of *HLA-B*, 138 alleles of *HLA-C*, 68 alleles of *HLA-DPB1*, 300 alleles of *HLA-DQB1* and 504 alleles of *HLA-DRB1* that were detected by high resolution genotyping. The remaining alleles presented with ambiguity and were not assessed.

Genotyping

Genomic DNA was isolated from peripheral blood using a standard phenol-chloroform technique. We used Sanger sequencing to analyze HLA class I and II. Conventional polymerase chain reaction was used to amplify the *HLA-A* gene (exons 2, 3 and 4); *HLA-B* gene (exons 2, 3 and 4); *HLA-C* gene (exons 2, 3 and 4); *HLA-DPB1* gene (exons 2, 3 and 4); *HLA-DRB1* gene (exon 2); and *HLA-DQB1* gene (exons 2 and 3). These exons encode the extracellular domains responsible for the peptide-binding grooves of HLA molecules[4]. These amplified fragments were directly sequenced in the forward direction using an ABI 3130 Avant Genetic Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). The sequences were compared by application-specific software (Assign SBT, Conexio-Genomics, Fremantle, Australia and HLA SBT U-Type 6.0 RUO by Life Technologies) to define the HLA class I and II alleles. The allelic frequencies were reported using a high-resolution level. The ambiguous HLA genotypes were not suitable for statistical analysis and excluded from each allele group.

Data analysis

Data were analyzed using SPSS statistics, version 19. The allelic frequencies, including homozygotes and heterozygotes of the HLA class I and class II, were compared using the chi-squared test with Yates' continuity correction. In all assays, statistical significance was determined at $p < 0.05$. These values were corrected by the Bonferroni method according to the number of alleles analyzed. The odds ratio (OR) was calculated and 95% confidence intervals (CI) were used to describe the strength of association between data.

Standard protocol approvals

This study was approved by the Ethical Committee for Research on Humans of the Hospital de Clínicas, Universidade Federal do Paraná, and was conducted after obtaining patient consent.

RESULTS

HLA class I

Of the 15 patients analyzed, nine showed ambiguous results for *HLA-A* alleles; six showed ambiguous results for *HLA-B* alleles and seven patients showed ambiguous results for *HLA-C* alleles. These patients were excluded.

HLA-A alleles

The most frequent alleles in the NMO group and the control group were **02:01* and **24:02*. Alleles **02:08* and **30:09* did not occur in the controls and the difference in that frequency was statistically significant, which became non-significant after the Bonferroni correction (Table 2).

Table 2. Frequency of HLA class I A alleles in NMOSD patients from southern Brazil.

Variable	NMOSD	Controls	p-value	OR (95% CI)	p^c
	6 patients, 12 alleles n (%)	192 individuals, 384 alleles n (%)			
HLA-A					
**02:01*	2 (16.6)	79 (20.5)	1.000	0.77 (0.17–3.66)	NS
**24:02*	2 (16.6)	38 (9.9)	0.3463	1.82 (0.38–8.62)	NS
**02:08*	1 (8.3)	0	0.0061		NS
**30:09*	1 (8.3)	0	0.0061		NS
Others					
Both groups #	6 (66.4)	140 (36.2)	NS §		NS
Control group †	0	127 (33.4)			

HLA: human leukocyte antigen; NMOSD: neuromyelitis optica spectrum disorders; p-value: value of p using the chi-squared test with Yates' continuity correction; OR: odds ratio; p^c: Probability after Bonferroni correction for 10 multiple comparisons for $p < 0.05$; NS: Non-significant. #: At least one allele was found in both groups: **01:01, *03:01, *24:03, *26:01, *66:01, *80:01*; † Alleles found only in controls (one or more), **01:02, *01:03, *02:02, *02:04, *02:05, *02:11, *02:20, *03:02, *11:01, *23:01, *23:05, *24:03, *25:01, *29:01, *29:02, *30:01, *30:02, *30:04, *31:01, *32:01, *33:01, *34:02, *38:02, *66:02, *68:01, *68:02, *69:01, *74:01, *74:03*. §: statistical analysis was done comparing each allele individually.

HLA-B alleles

The most frequent was **07:02*, which was also frequent in the controls. Alleles **08:04* and **35:04* did not appear in the controls and the difference in that frequency was statistically significant, which became non-significant after the Bonferroni correction (Table 3).

HLA-C alleles

Allele **04:01* was the most frequent in the NMO group and the control group. Allele **15:02* was rare in the controls and the difference in that frequency was statistically significant (p = 0.0287, OR 19.6 95% CI 1.67–229.70). The difference in frequency of alleles lost their significance after the Bonferroni correction (Table 4).

HLA class II

Of the 15 patients analyzed, three showed ambiguous results for *HLA-DPB1* and for *HLA-DQB1*. These patients were excluded.

HLA-DPB1 alleles

The most frequent alleles were *DPB1*04:02* and **04:01*, which were also frequent in the controls. The alleles *DPB1*17:01* and **16:01*, despite the increased OR, were not significant. The other alleles were not statistically significant when compared with the control group (Table 5).

HLA-DQB1 alleles

The most frequent alleles were *DQB1*05:01* and **02:01*, which were also frequent in the controls. The difference in frequency of other alleles were not statistically significant when compared with the control group (Table 6).

HLA-DRB1 alleles

The most frequent alleles were *DRB1*03:01*, **04:05*, **10:01* and **16:02*. Compared with the control group, these differences in frequency were statistically significant for **04:05* (p < 0.0001; OR 27.89; 95% CI 4.47-173.97) and **16:02*

Table 3. Frequency of HLA class I B alleles in NMOSD patients from southern Brazil.

Variable	NMOSD	Controls	p-value	OR	p^c
	9 patients, 18 alleles n (%)	231 individuals, 462 alleles n (%)		(95%CI)	
HLA-B					
**07:02*	3 (16.7)	31 (6.7)	0.2513	2.78 (0.76–10.12)	NS
**08:04*	1 (5.6)	0	0.0148		NS
**35:04*	1 (5.6)	0	0.0148		NS
Others					
Both groups #	13 (27.9)	125 (27.2)	NS §		NS
Control group †	0	306 (66.1)			

HLA: human leukocyte antigen; NMOSD: neuromyelitis optica spectrum disorders; p-value: value of p using the chi-squared test with Yates' continuity correction; OR: odds ratio; p^c: Probability after Bonferroni correction for 16 multiple comparisons for p < 0.05; NS: Non-significant. #: At least one allele was found in both groups: **08:01, *14:01, *15:04, *15:10, *18:01, *35:02, *35:20, *51:01, *52:01, *53:01, *55:01, *57:01, *57:03*. †: Alleles found only in controls (one or more), **07:05, *13:02, *14:02, *14:03, *15:01, *15:03, *15:08, *15:15, *15:17, *15:18, *15:20, *15:33, *15:39, *18:04, *18:07, *27:02, *27:05, *35:01, *35:03, *35:05, *35:06, *35:08; *35:10; *37:01; *38:01; *39:01; *39:03; *39:05; *39:09; *40:01; *40:02; *40:04, *40:05, *40:08, *40:11, *41:01, *41:02, *42:01, *42:02, *44:02, *44:03, *44:04, *44:05, *45:01, *46:01, *47:01, *48:01, *48:02, *48:03, *49:01, *50:01, *50:02, *51:05, *51:07, *56:01, *58:01, *58:02, *73:01, *78:01, *81:01, *82:01*. §: statistical analysis was done comparing each allele individually.

Table 4. Frequency of HLA class I C alleles in NMOSD patients from southern Brazil.

Variable	NMOSD	Controls	p-value	OR (95% CI)	p^c
	8 patients, 16 alleles n (%)	69 individuals, 138 alleles n (%)			
HLA C					
**04:01*	3 (18.7)	20 (14.5)	0.9348	1.36 (0.36-5.21)	NS
**15:02*	2 (12.5)	1 (0.7)	0.0232	19.6 (1.67-229.70)	NS
**06:02*	2 (12.5)	14 (10.1)	0.7701	1.26 (0.26-6.15)	NS
Others					
Both groups #	9 (56.3)	63 (45.6)	NS §		NS
Control group †	0	40 (29.1)			

HLA: human leukocyte antigen; NMOSD: neuromyelitis optica spectrum disorders; p-value: value of p using the chi-squared test with Yates' continuity correction; OR: odds ratio; p^c: Probability after Bonferroni correction for 12 multiple comparisons for p < 0.05; NS: Non-significant; #: At least one allele was found in both groups: **03:01, *03:03, *03:04, *04:07, *07:02, *08:02, *14:02*; †: Alleles found only in controls (one or more), **01:02, *02:02, *02:10, *03:02, *04:02, *05:01, *06:01, *06:05, *07:01, *07:04, *07:06, *12:01, *12:02, 12:03, *13:01, *15:05, *16:01, *16:02, *17:01*; §: statistical analysis was done comparing each allele individually.

Table 5. Frequency of HLA class II DPB1 in NMOSD patients and healthy controls from southern Brazil.

Variable	NMOSD	Controls	p-value	OR (95% CI)	p^c
	12 patients, 24 alleles n (%)	34 individuals, 68 alleles n (%)			
DPB1					
**04:02*	5 (20.8)	9 (13.2)	0.5752	1.72 (0.51–5.78)	NS
**04:01*	4 (16.7)	18 (26.5)	0.4904	0.55 (0.17–1.85)	NS
**17:01*	3 (12.5)	2 (2.9)	0.2105	4.71 (0.74–30.14)	NS
**03:01*	2 (8.3)	7 (10.3)	0.7810	0.79 (0.15–4.11)	NS
**16:01*	2 (8.3)	1 (1.5)	0.3376	6.09 (0.53–70.47)	NS
Others					
Both groups #	8 (33.4)	19 (27.9)	NS §		NS
Control group †	0	12 (17.7)			

HLA: human leukocyte antigen; NMOSD: neuromyelitis optica spectrum disorders; p-value: value of p using the chi-squared test with Yates' continuity correction; OR: odds ratio; p^c: Probability after Bonferroni correction for 13 multiple comparisons for $p < 0.05$; NS: Non-significant; #: At least one allele was found in both groups: **01:01, *02:01, *02:02, *05:01, *13:01, *15:01, *46:01, *100:01*. †: Alleles found only in controls (one or more), **06:01, *09:01, 11:01, *14:01, *19:01, *105:01*; §: statistical analysis was done comparing each allele individually.

Table 6. Frequency of HLA class II DQB1 alleles in NMOSD patients and healthy controls from southern Brazil.

Variable	NMOSD	Controls	p-value	OR (95% CI)	p^c
	12 patients, 24 alleles n (%)	150 individuals 300 alleles n (%)			
DQB1					
**05:01*	5 (20.8)	29 (9.7)	0.1702	2.46 (0.85–7.08)	NS
**02:01*	4 (16.7)	33 (11.0)	0.6126	1.62 (0.52–5.02)	NS
**03:01*	2 (8.3)	56 (18.7)	0.3202	0.40 (0.09–1.73)	NS
**03:02*	2 (8.3)	30 (10.0)	0.7923	0.82 (0.18–3.65)	NS
**03:19*	2 (8.3)	5 (1.7)	0.1521	5.36 (0.98–29.25)	NS
**04:02*	2 (8.3)	16 (5.3)	0.8773	1.61 (0.34–7.47)	NS
Others					
Both groups #	7 (37.6)	89 (29.7)	NS §		NS
Control group †	0	42 (13.9)			

HLA: human leukocyte antigen; NMOSD: neuromyelitis optica spectrum disorders; p: value of p using the chi-squared test with Yates' continuity correction; OR: odds ratio; p^c: Probability after Bonferroni correction for 13 multiple comparisons for $p < 0.05$; NS: Non-significant; # At least one allele was found in both groups: **03:03, *05:02, *05:03, *06:02, *06:03, *06:04, *06:30*; †: Alleles found only in controls (one or more), **02:02, *03:04, *03:19, *06:01, *06:09, *16:01*; §: statistical analysis was done comparing each allele individually.

(p = 0.0005; OR 13.89; 95% CI 2.96-65.19) (Table 7). The difference in frequency of other alleles were not statistically significant when compared with the control group.

DISCUSSION

In NMO, the frequency of the HLA class I alleles has only rarely been described[10]. This is the first study to investigate the HLA class I alleles in Brazilian NMO patients by high-resolution methods. Our most frequent HLA class I alleles were *A*02:01*, *A*24:02*, *B*07:02* and *C*04:01*. As the previously published data were collected using low-resolution techniques, we did not find any previous reports with sufficiently high-resolution methods to compare with our data[10].

The allele *HLA-A*24:02* was related to autoimmune type 1 diabetes, and more often found in optic neuritis patients and in primary Sjögren syndrome patients[23,27,28,29]. Our NMO patients with *HLA-A*24:02* did not present with any such diseases, except optic neuritis.

The alleles *HLA-B*07:02* and *HLA-C*04:01* were the most frequent alleles in our NMO patients, as well as in the control population.

The frequencies of some HLA class II alleles were similar to the previous profiles described in European populations[10,14,25].

In our patients, the HLA class II *DPB1*04:01, *04:02* and **17:01* alleles were the more frequent, but the differences in their frequencies were not significant compared with the control population. The frequency of allele *DPB1*04:01* was similar to that obtained in French Caucasian patients and in Asian NMO patients (Figure 1)[9,10,15,18]. However, the HLA class II *DPB1*05:01* allele showed a somewhat higher frequency in NMO Asian patients, but not in our patients (Figure 1)[9,10,15,18]. In addition, although the allele *DPB1*01:01* was previously

Table 7. Frequency of HLA class II DRB1 alleles in NMOSD patients and healthy controls from southern Brazil.

Variable	NMOSD	Controls	p-value	OR (95% CI)	p^c
	15 patients, 30 alleles n (%)	252 individuals, 504 alleles n (%)			
DRB1					
**03:01*	5 (16.7)	28 (5.6)	0.389	3.4 (1.21-9.55)	NS
**04:05*	3 (10.0)	2 (0.4)	< 0.0001	27.89 (4.47-173.97)	0.0016
**10:01*	3 (10.0)	22 (4.4)	0.3298	2.43 (0.69-8.64)	NS
**16:02*	3 (10.0)	4 (0.8)	0.0005	13.89 (2.96-65.19)	0.0085
**01:01*	2 (6.7)	20 (4.0)	0.8028	1.72 (0.38-7.77)	NS
**07:01*	2 (6.7)	88 (17.5)	0.1994	0.34 (0.08-1.44)	NS
**13:02*	2 (6.7)	15 (3.0)	0.5597	2.33 (0.51-10.69)	NS
Others					
Both groups #	10 (33.2)	194 (38.6)	NS §		NS
Control group †	0	131 (25.7)			

HLA: human leukocyte antigen; NMOSD: neuromyelitis optica spectrum disorders; p: value of p using the chi-squared test with Yates' continuity correction; OR: odds ratio; p^c: Probability after Bonferroni correction for 17 multiple comparisons for $p < 0.05$; NS: Non-significant; #: At least one allele was found in both groups: **04:02, 04:04, 08:01, 08:07, *11:01, *11:03, *11:04, *13:01, *14:01, *15:03*. †: Alleles found only in controls (one or more), **01:02, *01:03, *03:02, *03:13, *04:01, *04:03, *04:07, *04:08, *04:11, *08:02, *08:04, *09:01, *09:10, *11:02, *12:01, *12:02, *13:03, *13:56, *14:04, *14:06, *15:01,*15:02, *16:01*; §: statistical analysis was done comparing each allele individually.

reported as having increased susceptibility to NMO in a French population, this allele was not statistical significant in our study, as well as not found in an Asian population (Figure 1)[9,10,15,18]. The *HLA-DPB1*02:02*, **15:01*, **46:01* and **100:01* alleles occurred only in our NMO patients and this requires further investigation in other populations to confirm their real influence on the NMO susceptibility.

The HLA class II alleles *DQB1*02:01* and *DQB1*05:01* showed higher frequencies in our patients, but these differences were not statistically significant compared with our control population. Similar findings were described in the French, Muslim Arabs in Israel, and southeastern Brazilian patients (Figure 2)[10,20,21]. The *HLA-DQB1*06:30* allele was found in one NMO patient, but not in the controls, and this discovery may suggest an increased susceptibility to the disease. In French Afro-Caribbean patients, the allele *DQB1*06* was statistically correlated with multiple sclerosis but not with NMO patients[16].

In our patients, the *DRB1*03:01* was more frequent than in the control group, which was similar to the southeastern Brazilian, Chinese and Japanese studies but different to the Muslim Arabs in Israel study (Figure 3)[9,15,18,20,21,22]. The difference in frequency of the *DRB1*04:05* allele showed a positive correlation (increased susceptibility) in our study; however, previous studies in Asian populations revealed a negative correlation (decreased susceptibility) in NMO patients (Figure 3)[9,15,18,20]. This same allele was associated with other autoimmune diseases in Asian populations[30,31]. In addition, a positive relationship of the allele **16:02* was found in our study, which was similar to others studies in Asian populations (Figure 3)[9,15,18]. We did not find any NMO patients with allele **15:01*, which is quite common in multiple sclerosis and frequent in our population[22,32].

To date, there have been only three studies performed with Brazilian patients, who had a different ethnic background when compared with southern Brazil because the recruited patients were from southeastern Brazil[11,12,21]. The first Brazilian study showed that the most frequent HLA class II allele was the *DRB1*03* allele in mulatto-designated NMO patients[11]. This allele frequency was similar to our study, but when compared with the control group from southern Brazil, the difference was not statistically significant. In the second Brazilian study, which was carried out in Afro-descendent and Caucasian patients, no association was found between NMO and the extended haplotype *DRB1*15:01-DQA1*01:02-DQB1*06:02* or their alleles[12]. The third Brazilian study was the most recent, which showed similar findings of the allele *DRB1*03:01* to our NMO patients from southern Brazil[21].

In our study, the small sample size influences the analysis of some HLA profiles, which was a limitation in this study. Additionally, we could speculate that the small size of our cohort, which was aggravated by the exclusion of some patients with an ambiguous genotype that was not suitable for statistical analysis, influenced the distribution of the HLA genotype. However, our findings confirmed that the southern Brazilian population has a different ethnic background than southeastern Brazilian populations.

In conclusion, our study suggested a different ethnic background of NMO in southern Brazilian patients than in other Brazilian populations. Currently, the southern Brazilian population has a multi-ethnic nature and their HLA profile may be different from the original European descendants. In our NMO patients, an European profile was expected, but the HLA genotyping resembled European and Asian populations, revealing the current mixing of the HLA profile of the southern Brazilian population. In addition, some new HLA genotypes were associated with increased susceptibility to NMO in our patients, particularly for some HLA class I and *HLA-DRB1*04:05* and **16:02*.

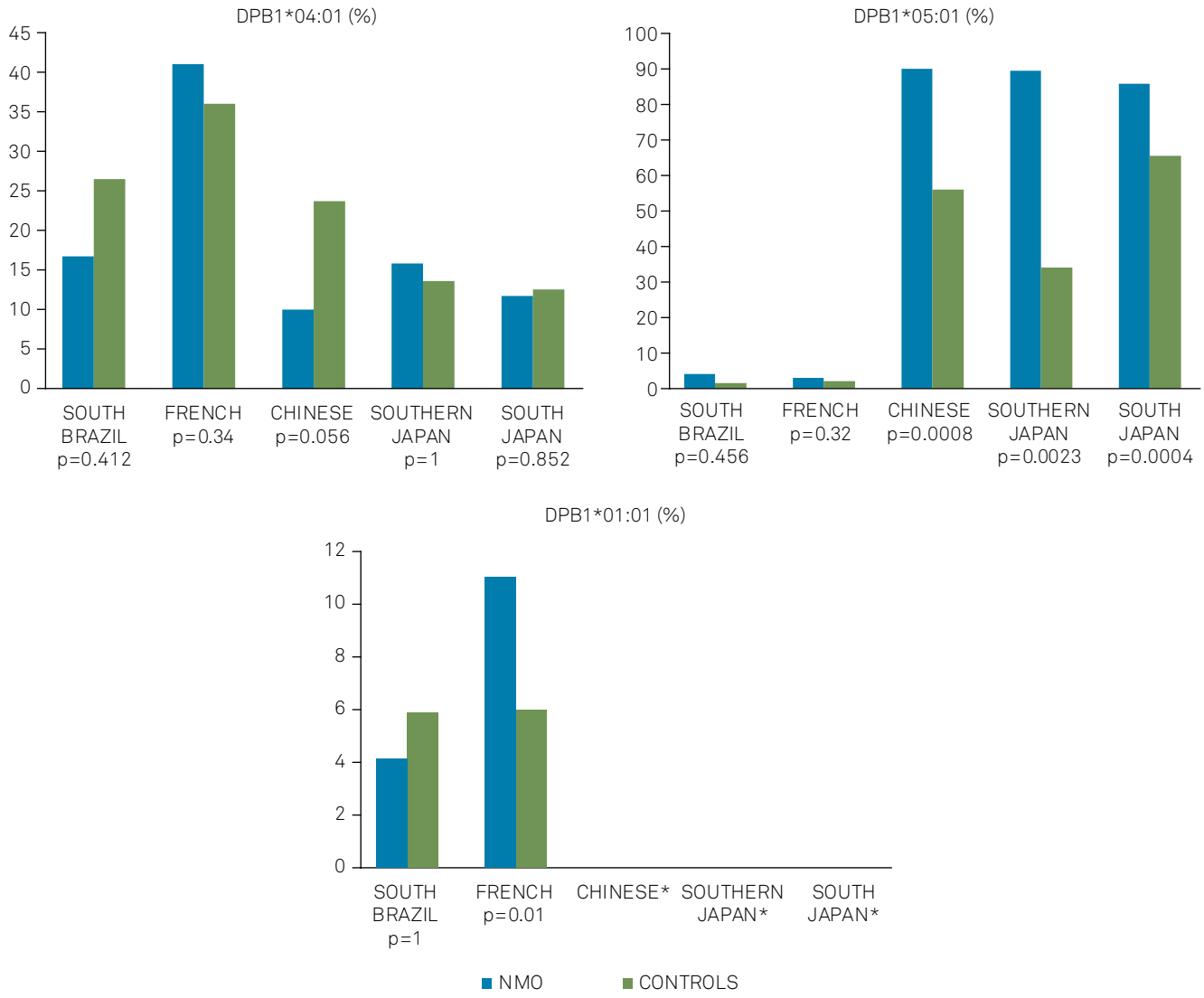

Figure 1. The frequency of **04:01, *05:01* and **01:01* alleles of the HLA-DPB1 comparing southern Brazil (present study), French[10], China[15], southern Japan[9] and south Japan[18] populations. #: allele was not found in patients or controls of this study.

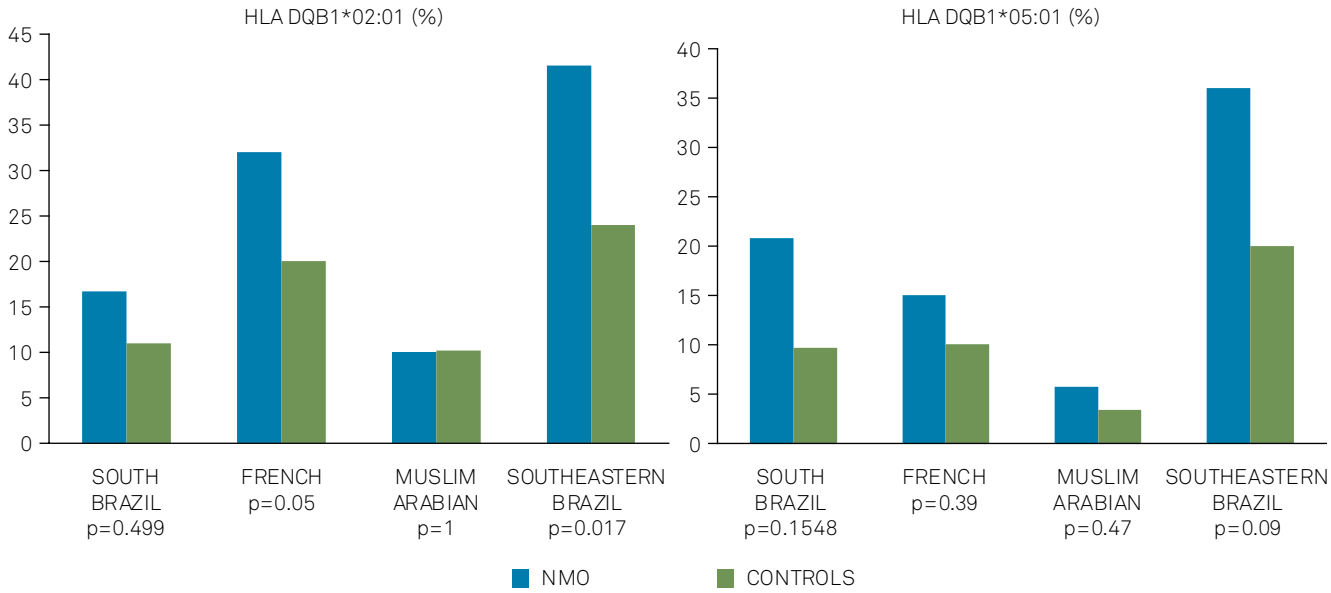

Figure 2. The frequency of **02:01* and **05:01* alleles of the HLA-DQB1 comparing southern Brazil (present study), French[10], China[15], Muslim Arabian (Israel)[20] and southeastern Brazil[21] populations.

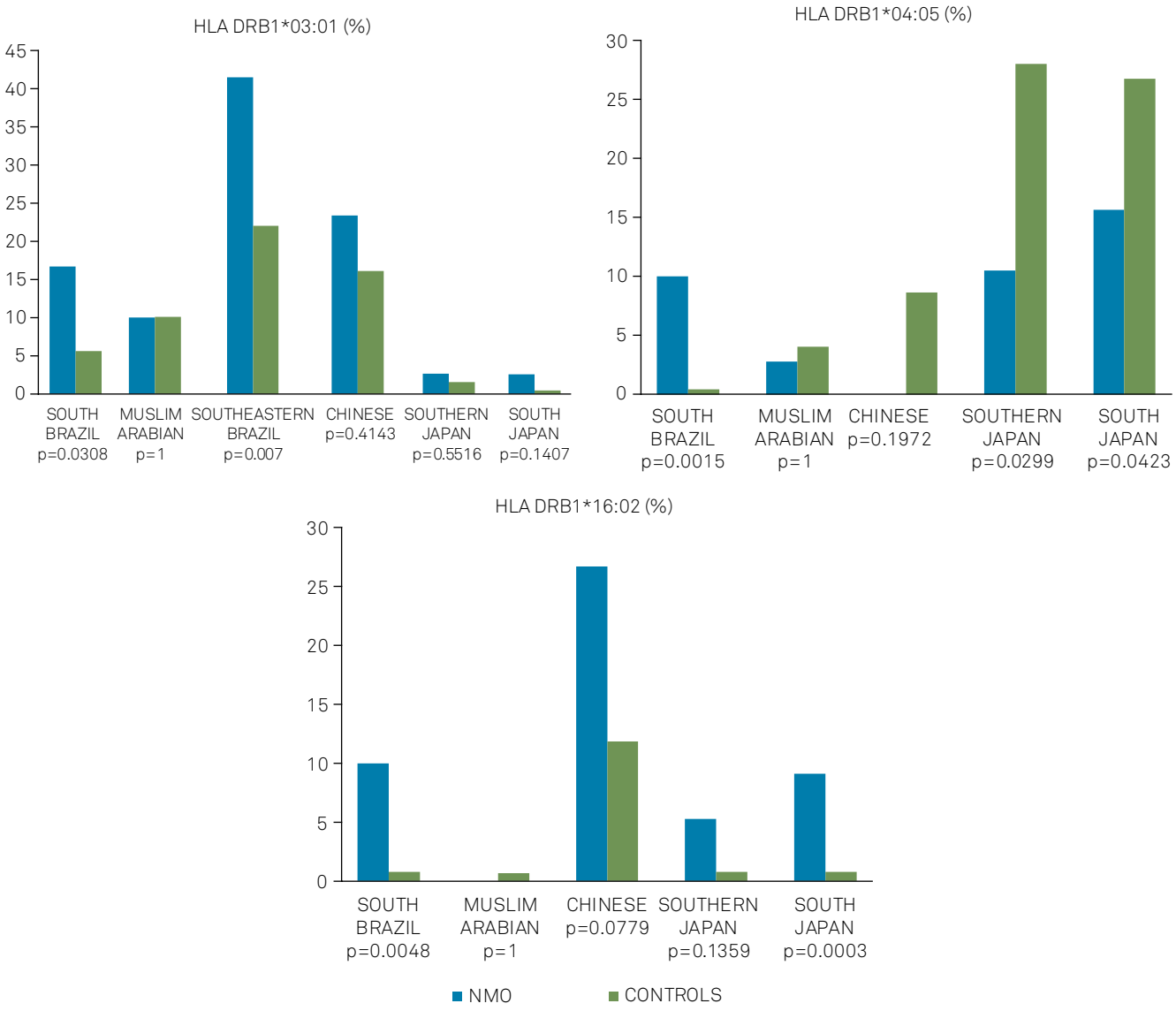

Figure 3. The frequency of **03:01, *04:05* and **16:02* alleles of the HLA-DRB1 comparing southern Brazil (present study), Muslim Arabian (Israel)[20], southeastern Brazil[21], China[15], southern Japan[9] and south Japan[18] populations.

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