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ARTICLE

Multiple sclerosis: disease modifying therapy and the human leukocyte antigen

Esclerose múltipla: terapêutica modificadora da doença e antígenos leucocitários humanos

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ABSTRACT

Objective: To investigate the potential relationship between the human leukocyte antigen (HLA) type (class I and II) and the response to several disease-modifying therapies (DMTs) in patients with multiple sclerosis (MS). **Methods:** We analyzed clinical data of 87 patients with MS at the beginning and end of each type of DMT including the disease duration, Expanded Disability Status Scale and Multiple Sclerosis Severity Score (MSSS). Genotyping of HLA-DRB1, HLA-DPB1, HLA-DQB1, HLA-A, HLA-B and HLA-C alleles were identified using high-resolution techniques. Statistical correlation between the HLA type and response to DMTs was done using the initial and final MSSS. **Results:** Statistical relationships ($p < 0.05$) were found for only 15 of 245 alleles tested. There was a reduction in the MSSS for patients treated with corticosteroids (DRB1*15:01, DPB1*04:01, DQB1*02:01 and DQB1*03:01), azathioprine (DRB1*03:01, DPB1*04:01, DQB1*03:02, DQB1*06:02, HLA-C*07:02), interferon β-1a 22 mcg (DRB1*11:04, DQB1*03:01 and DQB1*03:02), interferon β-1a 30 mcg (DPB1*02:01, HLA-C*05:01) and interferon β-1b (DQB1*02:01). **Conclusion:** These findings suggest a few relationships between the HLA and response to DMTs in the disability for some types of HLA class I and II alleles in a specific subset of MS patients.

Keywords: Multiple sclerosis; biomarkers; pharmacogenetics; therapeutics.

RESUMO

Objetivo: Investigação da possível relação entre os tipos dos antígenos leucocitários humanos (HLA) classes I e II e a reposta a diversas terapêuticas modificadores da doença na incapacidade (DMT) da esclerose múltipla (MS). **Métodos:** Foram estudados os dados clínicos de 87 pacientes com MS no início e no final de cada de cada DMT, incluindo o tempo de doença, EDSS e MSSS. Através de técnicas de genotipagem de alta resolução, foram identificados os alelos dos HLA-DRB1, HLA-DPB1, HLA-DQB1, HLA-A, HLA-B e HLA-C. Foram realizados estudos estatísticos entre os tipos de HLA e a resposta às DMT, utilizando os valores iniciais e finais do MSSS. **Resultados:** Foram encontradas relações estatísticas ($p < 0.05$) para somente 15 alelos de 245 analisados. Houve redução dos valores do MSSS em pacientes tratados com corticosteroides (DRB1*15:01, DPB1*04:01, DQB1*02:01 e 03:01), azatioprina (DRB1*03:01, DPB1*04:01, DQB1*06:02, DQB1*03:02, HLA-C*07:02), interferon β-1a 22 mcg (DRB1*11:04, DQB1*03:01 e 03:02), interferon β-1a 30 mcg (DPB1*02:01, HLA-C*05:01) e interferon β-1b (DQB1*02:01). **Conclusão:** Os dados sugerem poucas relações entre os alguns tipos de HLA classe I e II com a resposta às DMT na incapacidade em grupos específicos de pacientes com MS.

Palavras-chave: Esclerose múltipla; biomarcadores; farmacogenética; terapêutica.

Treatment of multiple sclerosis (MS) has progressed significantly in recent years and now includes immunosuppressants, immunomodulators, biologics and, more recently, medications that interfere with the release or maturation of lymphocytes. These medications have been shown to reduce the number of relapses, but the results in terms of reduced disability have been modest as most of the studies carried out were generally restricted to two years[1,2].

Since the introduction of immunomodulators to treat MS, the response has been found to vary from patient to patient and with the research center where the study was conducted[3]. To find a factor that influences the evolution of MS in patients treated with disease modifying therapies (DMTs), several

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attempts have been made to identify a relationship with the patient's immunogenetic profile, e.g. the human leukocyte antigen (HLA) type, but without success[4,5,6,7,8]. The HLA are glycoproteins present on the surface of lymphocytes of the major histocompatibility complex (MHC) and are responsible for the initiation of the immunological response for an antigen. The MHC expresses three different antigen-present cell classes of HLA. The MHC class I (HLA A, B and C) molecules present the peptides from pathogens, such as a virus, to CD8 cytotoxic cells. The CD4 T cells recognize the MHC class II (HLA DRB1, DPB1 and DQB1) in other cells of the immune system stimulating the B cells to produce antibodies[9].

The genes that codify these HLA cell proteins are highly polymorphic and variable, according to the population studied, with some specific types related to MS[9,10]. The failure to find a relationship between the HLA type and response to DMTs may be due to the variation in the immunogenetic profiles of the populations studied and the different techniques used in the HLA typing has previously been reported[10]. However, some studies have shown an association between the presence of neutralizing antibodies and HLA alleles such as HLA-DRB1*03:01, HLA-DRB1*04:04, HLA-DRB1*11:04[11] and the HLA-DRB1*07:01-DQB1*02:02 haplotype[12]. One study found a relationship between a better response to interferon β and an increased frequency of HLA-DRB1*04, as well as between the HLA-A*03-B*44-DRB1*04 haplotype and decreased frequency of HLA-B*15[13]. Another study found an association between the response to glatiramer acetate and HLA-DQB2, as well as other genes[14].

To date, the relationship between the response to DMTs and the patient's HLA type, disease duration, age, disability and duration of DMT has not been well established. For this reason, in this study we investigated the associations between the HLA (class I and II) profile and disability progression (therapeutic response) in a group of MS patients treated with DMTs in a real-world outpatient clinic.

METHODS

We analyzed 87 patients (57 females and 30 males, 84 whites and three non-whites) with a diagnosis of relapsing-remitting MS based on the 2010 and 2013 revisions to the McDonald criteria[15,16]. The patients were treated at the Demyelinating Disease Outpatient Clinic of the Neurology Service at the Hospital de Clínicas, Federal University of Paraná, Curitiba, Brazil. The MS patients who fulfilled the following criteria were included: 1) patient who had complete medical records available with information on the DMT used and disease duration at the beginning and end of each type of treatment; 2) patients must have had at least six months of therapy with only one DMT; 3) patients with more than one DMT at same time were excluded, excepted for the use of corticosteroid during exacerbation, but in these instances, they were placed only in the DMTs already in use, not in the corticosteroid group, and 4) patients with an available assessment on the Expanded Disability Status Scale (EDSS)[17] or sufficient data in the patient's records to allow their disability to be graded at the beginning and end of each treatment. The MS patients who had incomplete medical records or conflicting data, or who had not been treated with DMTs were excluded (Table 1).

Data was recorded at the beginning and end of each type of treatment, including the disease duration and EDSS score, which allowed calculation of the Multiple Sclerosis Severity Score (MSSS)[18] and correlation with the DMT used.

The MSSS score was obtained by the correlation of the degree of disability (EDSS) and duration of disease[18]. The patients with less than one year, were placed in the MSSS as year one and the score was correlated with treatment duration and the HLA genotype. The change in the MSSS between the beginning and end of each type of treatment (MSSS initial and MSSS final) was compared using the paired-sample Student's t-test (t)[19] or Wilcoxon signed-ranks test (T)[20], with SPSS-19 statistical software.

We chose a high-resolution technique for DNA analysis of the HLA system, because the old technique with low resolution shows only the serological result of the protein like the alleles group, but not the specific HLA protein[21,22].

Briefly, as we described previously[10], the genomic DNA was extracted from blood previously stored at -70°C, 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). The PCR was performed separately for each exon of these different genes using a conventional method with *Taq* DNA polymerase (Abbott Molecular Diagnostics) and a sequence based typing (SBT) kit (AlleleSEQR-SBT) (Atria Genetics) following the manufacturers' instructions. The PCR products were purified by ExoSAP-IT® (USB, Cleveland, Ohio). The purified 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)[10].

Of the 87 patients studied, 79 were tested for HLA-DRB1, 79 for HLA-DPB1, 77 for HLA-DQB1, 54 for HLA-A, 58 for HLA-B and 78 for HLA-C. Since each patient had two alleles,

Table 1. Disease-modifying therapy and HLA. Gender, age, duration of symptoms, EDSS, MSSS and HLA alleles.

HLA	DRB1*	DPB1*	DQB1*	A*	B*	C*
N° alleles tested	50	28	36	38	55	36
Female (No. of patients)	53	50	49	35	36	49
Male (No. of patients)	26	29	28	19	22	29
White	76	76	74	52	55	75
Non-white	3	3	3	2	3	3
Age at treatment (years)						
Mean	33.88 ± 10.39	34.86 ± 10.57	34.30 ± 10.66	35.30 ± 10.50	36.26 ± 10.48	34.33 ± 10.59
Median	33.00 (12–60)	35.00 (12–61)	34.00 (12–63)	36.00 (12–60)	35.00 (12–63)	34.00 (12–63)
Age at onset of symptoms (years)						
Mean	29.00 ± 9.59	29.95 ± 9.99	29.62 ± 10.00	30.56 ± 9.81	29.67 ± 9.99	29.86 ± 10.04
Median	29.00 (12–50)	30.00 (12–61)	29.00 (12–61)	30.50 (12–50)	29.50 (12–61)	30.00 (12–61)
Duration of symptoms (months)						
Mean	64.44 ± 71.13	60.09 ± 71.23	61.75 ± 71.64	65.14 ± 77.61	67.53 ± 78.90	62.27 ± 71.52
Median	48.00 (0–373)	36.00 (0–373)	39.00 (0–373)	39.00 (0–373)	47.00 (0–373)	40.50 (0–373)
Duration of treatment (months)						
Mean	46.17 ± 46.13	46.25 ± 46.69	45.63 ± 46.55	46.25 ± 47.78	49.51 ± 51.12	46.85 ± 47.31
Median	30.00 (6–276)	30.00 (6–276)	28.00 (6–276)	29.00 (6–276)	30.00 (96–276)	29.50 (6–276)
EDSS (mean)						
Initial	2.62 ± 1.92	2.60 ± 1.90	2.60 ± 1.83	2.48 ± 1.72	2.46 ± 1.87	2.58 ± 1.83
Final	3.07 ± 2.26	3.00 ± 2.25	2.96 ± 2.15	2.85 ± 2.06	2.88 ± 2.20	3.00 ± 2.17
t p=	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
MSSS (mean)						
Initial	4.59 ± 3.02	4.63 ± 3.30	4.62 ± 2.96	4.42 ± 2.90	4.28 ± 3.00	4.57 ± 2.96
Final	4.21 ± 3.04	4.19 ± 3.02	4.11 ± 2.92	3.93 ± 2.87	3.55 ± 2.93	4.16 ± 2.95
t p=	0.002	0.001	< 0.001	0.002	0.005	0.001

No.: number; EDSS: Expanded Disability Status Scale; MSSS: Multiple Sclerosis Severity Score; *t*: paired-sample Student's t-test.

the analysis comprised 158 alleles of HLA-DRB1, 158 of HLA-DPB1, 154 of HLA-DQB1, 108 of HLA-A, 116 of HLA-B and 156 of HLA-C (Table 1). Some patients were not tested for all alleles because of technical problems, and others yielded inconclusive results due to ambiguities.

Twelve different DMTs were used, and most patients were treated with more than one (Table 2). When more than one DMT was used by the same patient at a different time, each treatment was considered a single case during the period of the single drug. To be included in a specific group of DMT, we chose patients using only one DMT for at least six months (Table 2). If two drugs were used at same time, the patient was excluded from both groups of DMTs, except for corticosteroids during the relapses. If a corticosteroid was used concomitantly with another DMT during relapses, the patient was not included in the corticosteroid group but placed into the specific DMT being used. All patients in the corticosteroid group were not using any other DMT during the follow-up period. The following DMTs were used: corticosteroids, as soon as the signs or symptoms of relapses started in the first 24 hours (prednisone 80-140 mg, q.d. orally, followed by 1 mg prednisone/kg/day orally for 15 days and progressive withdrawal in one to two months or methylprednisolone 1.0 g IV for three or five days) without any DMT during the follow-up period; azathioprine 2-3 mg/kg/day orally and a progressive dosage increase until mean corpuscular volume was above 100 fL; interferon β-1a 22 mcg t.i.w., subcutaneous (subcut); interferon β-1a 44 mcg t.i.w., subcut; interferon β-1a 30 mcg IM q.o.w.; interferon β-1b 0.25 mg q.o.d, subcut; glatiramer acetate 20 mg q.d., subcut; mitoxantrone 120 mg total dose in 18 months, IV; natalizumab 300 mg, q.m. IV; methotrexate 7.5-10 mg/week; fingolimod 0.5 mg, q.d., orally; or teriflunomide 14 mg, q.d., orally (Table 2).

The study was approved by the Ethics Committee for Research with Humans at the Hospital de Clínicas, Federal University of Paraná (CAAE: 0120.0.208.000-06, CEP: 1279.127/2006-09). All patients agreed to participate and signed a voluntary consent form.

RESULTS

The mean patient age was 34.08 ± 10.59 years, median 34.00 (ranging from 12 to 63 years) at the beginning of the DMT, and the mean treatment time was 46.18 ± 46.03 months, median 30 (ranging from 6 to 276 months).

The mean EDSS score was 2.53 ± 1.87, median 2.0 (ranging from 0 to 7.5) at the beginning of the DMT and 2.93 ± 2.22, median 2.50 (ranging from 0 to 8.5) at the end, indicating progression of disability ($p < 0.001$).

The MSSS reduced in the majority of the patients for all the HLA alleles tested in all kinds of treatment (Table 3).

Table 2. HLA classes and number of patients on each type of disease modifying therapy.

Type of treatment	HLA-DRB1* (N)	HLA-DPB1* (N)	HLA-DQB1* (N)	HLA-A* (N)	HLA-B* (N)	HLA-C* (N)
Corticosteroids	70	74	70	44	54	74
Duration	60.43 (7–276)	56.27 (7–276)	57.66 (7–276)	64.32 (12–276)	65.07 (12-276)	57.46 (7-276)
MSSS Initial	4.62 (0.17–9.09)	4.37 (0.17–9.09)	4.58 (0.35–9.09)	4.14 (0.35–9.09)	4.21 (0.35-9.09)	4.37 (0.17-9.09)
MSSS Final	3.55 (0.04–9.52)	3.59 (0.04–9.52)	3.59 (0.04–9.52)	2.83 (0.04–8.34)	3.33 (0.04-9.55)	3.63 (0.04-9.52)
t p =	0.001	0.009	0.002	0.002	0.017	0.011
Azathioprine	44	44	48	30	38	48
Duration	66.86 (6–216)	66.36 (6–216)	63.88 (6–216)	73.80 (6–216)	67.47 (6-216)	69.13 (6-216)
MSSS Initial	3.97 (0.35–9.93)	4.32 (0.35–9.93)	4.26 (0.35–9.93)	4.05 (0.35–9.93)	4.07 (0.35-9.35)	3.95 (0.35-9.93)
MSSS Final	3.85 (0.30–9.65)	3.73 (0.10–9.65)	3.74 (0.30–9.65)	3.62 (0.35–9.65)	3.47 (0.30-8.54)	3.55 (0.10-9.65)
t p =	0.570	0.010	0.016	0.162	0.023	0.076
Interferon β-1a 22 mcg	52	52	52	34	38	52
Duration	33.85 (6–102)	42.27 (6–147)	43.85 (6–147)	35.65 (6–101)	38.58 (6-102)	43.85 (6-147)
MSSS Initial	4.44 (0.32–8.64)	4.57 (0.32–8.64)	4.48 (0.32–8.64)	4.66 (0.32–8.64)	3.56 (0.32-8.58)	4.48 (0.32-8.64)
MSSS Final	4.74 (0.26–9.59)	4.63 (0.26–9.59)	4.39 (0.26–9.08)	4.88 (0.26–9.59)	4.13 (0.26-9.08)	4.39 (0.26-9.08)
t p =	0.433	0.896	0.828	0.646	0.252	0.828
Interferon β-1a 44 mcg	28	24	24	14	12	26
Duration	35.57 (9–96)	35.58 (9–96)	37.67 (9–96)	34.71 (18–96)	36.50 (9-96)	35.08 (9-96)
MSSS Initial	4.04 (0.26–7.98)	3.57 (0.26–7.98)	4.53 (0.26–7.98)	4.76 (0.26–7.98)	3.75 (0.26-7.98)	4.20 (0.26-7.98)
MSSS Final	3.67 (0.17–8.24)	2.96 (0.17–7.32)	4.24 (0.17–8.24)	4.27 (0.17–8.24)	3.64 (0.17-7.32)	3.86 (0.17-8.24)
t p =	0.182	0.033	0.371	0.289	0.646	0.262
Interferon β-1a 30 mcg	32	30	36	20	18	34
Duration	39.13 (6–90)	31.87 (8–80)	32.61 (6–90)	35.10 (12–80)	35.67 (12-80)	36.24 (6-90)
MSSS Initial	3.28 (0.30–7.32)	4.01 (0.30–7.32)	3.83 (0.30–7.32)	3.63 (1.28–7.32)	3.09 (0.30-7.32)	3.44 (0.30-7.32)
MSSS Final	2.96 (0.21–7.75)	3.42 (0.21–7.75)	3.05 (0.21–7.75)	3.57 (0.21–7.65)	2.78 (0.21-6.24)	3.02 (0.21-7.75)
t p =	0.443	0.245	0.079	0.917	0.436	0.328
Interferon β-1b	42	46	38	32	32	40
Duration	40.43 (7–132)	54.13 (7–132)	52.74 (7–132)	43.94 (7–132)	53.94 (7-132)	51.20 (7-132
MSSS Initial	4.69 (0.30–9.63)	4.50 (0.30–9.63)	4.58 (0.30–9.63)	4.45 (0.30–9.63)	4.32 (0.30-9.63)	4.55 (0.35-9.63)
MSSS Final	4.44 (0.25–8.83)	4.21 (0.25–8.83)	4.22 (0.25–8.83)	3.81 (0.25–8.83)	3.76 (0.25-8.83)	4.35 (0.32-8.83)
t p =	0.468	0.370	0.346	0.111	0.167	0.575
Glatiramer Acetate	42	36	38	32	28	36
Duration	36.24 (12–114)	36.22 (12–114)	33.26 (12–114)	31.06 (12–72)	38.57 (12-114)	32.94 (12-114)
MSSS Initial	4.72 (0.25–9.59)	4.94 (0.25–9.59)	4.46 (0.25–8.70)	4.52 (0.25–9.59)	4.51 (0.25-8.70)	4.95 (0.45-8.70)
MSSS Final	3.97 (0.17–8.50)	4.39 (0.17–8.50)	4.03 (0.17–8.38)	4.10 (0.17–8.50)	3.52 (0.17-8.38)	4.38 (0.21-8.38)
t p =	0.019	0.189	0.277	0.094	0.036	0.172
Mitoxantrone	16	16	14	6	10	16
Duration	19.50 (6–30)	19.50 (6–30)	18.43 (6–30)	21.33 (12–30)	20.80 (12-30)	19.50 (6-30)
MSSS Initial	8.11 (6.61–9.80)	8.11 (6.61–9.80)	8.01 (6.61–9.80)	7.47 (6.61–8.83)	7.81 (6.61-9.80)	8.11 (6.61-9.80)
MSSS Final	8.27 (6.14–9.95)	8.27 (6.14–9.95)	8.17 (6.14–9.95)	7.14 (6.14–8;31)	8.11 (6.14-9.95)	8.27 (6.14-9.95)
t p =	0.610	0.610	0.638	0.595	0.538	0.610
Natalizumab	6	4	6	4	4	6
Duration	21.33 (7–29)	18.00 (7–29)	21.33 (7–29)	18.00 (7–29)	18.00 (7-29)	21.33 (7-29)
MSSS Initial	6.92 (5.79–8.38)	7.08 (5.79–8.38)	6.92 (5.79–8.38)	7.08 (5.79–8.38)	7.08 (5.79-8.38)	6.92 (5.79-8.38)
MSSS Final	4.90 (2.10–7.33)	4.71 (2.10–7.33)	4.90 (2.10–7.33)	4.71 (2.10–7.33)	4.71 (2.10-7.33)	4.90 (2.10-7.33)
T p =	0.026	0.063	0.026	0.063	0.063	0.026
Methotrexate	4	4	4	2	4	4
Duration	18.50 (16–21)	18.50 (16–21)	18.50 (16–21)	16.00 (16–16)	18.50 (16-21)	18.50 (16-21)
MSSS Initial	7.77 (6.00–9.55)	7.77 (6.00–9.55)	7.77 (6.00–9.55)	6.00 (6.00–6.00)	7.77 (6-9.55)	7.77 (6.00-9.55)
MSSS Final	9.00 (8.92–9.08)	9.00 (8.92–9.08)	9.00 (8.92–9.08)	9.08 (9.08–9.08)	9.00 (8.92-9.08)	9.00 (8.92-9.08)
t p =	0.458	0.458	0.458	0.157	0.458	458
Fingolimod	0	0	2	2	0	0
Duration	–	–	20.00 (20–20)	20.00 (20–20)	–	–
MSSS Initial	–	–	1.04 (1.04–1.04)	1.04 (1.04–1.04)	–	–
MSSS Final	–	–	0.21 (0.21–0.21)	0.21 (0.21–0.21)	–	–
t p =	–	–	0.157	0.157	–	–
Teriflunomide	2	2	2	2	0	0
Duration	6.00 (6–6)	6.00 (6–6)	6.00 (6–6)	6.00 (6–6)	–	–
MSSS Initial	3.17 (3.17–3.17)	3.17 (3.17–3.17)	3.17 (3.17–3.17)	3.17 (3.17–3.17)	–	–
MSSS Final	4.96 (4.96–4.96)	4.96 (4.96–4.96)	4.96 (4.96–4.96)	4.96 (4.96–4.96)	–	–
t p =	0.157	0.157	0.157	0.157	–	–

N: number of patients; Duration: mean treatment duration (Months); MSSS: Multiple Sclerosis Severity Score; Mean (minimum - maximum). -: The correlation and *t* could not be computed because the standard error of the difference is 0; *t*: paired-sample Student's t-test. *T*: Wilcoxon signed-ranks test.

The mean MSSS was 4.51 ± 3.01, median 4.30 (ranging from 0.17 to 9.93) at the beginning of the DMT and 4.06 ± 3.01 (ranging from 0.04 to 9.95) at the end. A reduction in the MSSS was observed when all the patients were analyzed together, suggesting a stabilization or improvement of the disability ($p < 0.001$). Most of the patients had a decrease in the MSSS despite the type of HLA or DMT, showing a beneficial effect of the therapy, but only few reached a statistically significant level (Table 3).

Some patients (two alleles) were treated with more than one DMT (total 1,794 occurrences comparing the MSSS between the initial and final score). The reduction of the MSSS occurred with most of the DMTs and specific alleles, such as HLA-DRB1 in 210/337, HLA-DPB1 in 204/331, HLA-DQB1 214/333, HLA-A 142/221, HLA-B 150/237 and HLA-C 204/335 (Table 3). However, the statistical relationships between the MSSS, HLA allele (subtypes of DRB1, DPB1, DQB1, A, B and C) and the DMT were significant ($p < 0.05$) for only 15/245 specific alleles with reduction of the MSSS (Table 4).

We found a statistically significant reduction in the MSSS, suggesting improvement of the disability for the following alleles: HLA-DRB1*15:01 (7/7), DPB1*04:01 (13/16), DQB1*02:01 (5/5) and DQB1*03:01 (8/8) treated with

Table 3. Number of patients with MSSS reduction and the most frequent HLA types in all DMT groups.

HLA Alleles	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R
DRB1*	**All**		**15:01**		**07:01**		**03:01**		**13:02**		**11:04**		**04:04**		**13:01**		**16:01**		**14:01**		**11:01**	
Number of patients	337	210	40	29	32	19	32	19	22	7	17	10	14	12	13	9	13	10	11	8	11	9
DPB1*	**All**		**04:01**		**02:01**		**04:02**		**03:01**		**10:01**		**23:01**		**01:01**		**14:01**		**05:01**		**13:01**	
Number of patients	331	204	79	50	32	20	31	16	27	14	24	12	16	12	19	11	10	5	10	8	8	3
DQB1*	**All**		**06:02**		**03:02**		**03:01**		**02:01**		**04:02**		**05:01**		**05:03**		**06:03**		**02:02**		**06:02**	
Number of patients	333	214	44	31	47	26	34	24	30	21	25	14	24	11	15	10	13	7	12	6	12	10
HLA-A*	**All**		**24:02**		**03:01**		**02:01**		**01:01**		**23:01**		**11:01**		**68:02**		**68:01**		**32:01**		**25:01**	
Number of patients	221	142	31	21	30	17	28	16	24	14	12	8	9	4	9	7	8	6	7	6	6	4
HLA-B*	**All**		**51:01**		**35:01**		**07:02**		**14:02**		**44:02**		**49:01**		**14:01**		**14:06**		**35:03**		**40:02**	
Number of patients	237	150	28	20	23	15	19	12	12	5	9	4	7	3	7	3	6	5	7	5	6	3
HLA-C*	**All**		**04:01**		**07:02**		**07:01**		**08:02**		**05:01**		**06:02**		**03:04**		**03:03**		**01:02**		**15:02**	
Number of patients	335	204	55	33	41	26	24	13	26	15	24	15	20	11	16	9	11	7	12	5	11	9

DMT: Disease Modifying Therapy; MSSS: Multiple Sclerosis Severity Score; T: total number of patients treated with DMT; R: number of patients with a reduction in MSSS.

Table 4. HLA alleles with a statistically-significant relationship with the DMT.

DMT	Number patients*/Total	Age (years)	Duration treatment** (months)	MSSS initial***	MSSS final***	p = *T*
HLA alleles*						
Corticosteroids						
DRB1*15:01	7/74	29.43 ± 9.97	96.29 (12-278)	5.99 ± 2.62	2.74 ± 1.85	0.018
DPB1*04:01	16/74	32.75 ± 9.48	90.81 (12-276)	4.55 ± 3.20	2.73 ± 2.84	0.023
DQB1*02:01	5/70	34.40 ± 4.27	78.20 (20-218)	4.87 ± 1.66	2.79 ± 1.24	0.043
DQB1*03:01	8/70	35.13 ± 12.03	82.25 (17-128)	5.00 ± 3.76	1.96 ± 1.54	0.011
Azathioprine						
DRB1*03:01	5/44	43.40 ± 8.23	70.00 (6-144)	7.73 ± 3.02	6.94 ± 3.65	0.043
DPB1*04:01	7/44	40.71 ± 5.34	86.29 (19-144)	4.35 ± 4.10	2.68 ± 2.30	0.042
DQB1*03:02	7/48	33.00 ± 14.90	25.71 (6-67)	3.58 ± 2.59	2.64 ± 2.43	0.018
DQB1*06:02	7/48	44.57 ± 11.07	94.71 (18-144)	6.83 ± 3.45	5.40 ± 3.82	0.018
C*07:02	5/48	38.60 ± 6.76	93.40 (21-144)	5.74 ± 4.23	3.88 ± 3.14	0.043
Interferon β-1a 22 µg						
DRB1*11:04	6/52	25.17 ± 11.42	48.33 (20-80)	6.80 ± 1.83	5.24 ± 3.11	0.027
DQB1*03:01	5/52	28.80 ± 10.96	50.60 (20-80)	6.10 ± 2.18	4.82 ± 2.82	0.043
DQB1*03:02	5/52	30.00 ± 8.74	29.60 (12-50)	4.01 ± 3.17	3.42 ± 3.17	0.042
Interferon β-1a 30 µg						
DPB1*02:01	5/30	42.20 ± 18.78	33.80 (22-49)	3.29 ± 1.00	2.03 ± 1.11	0.042
C*05:01	5/34	35.40 ± 6.30	40.40 (6-90)	2.18 ± 1.37	1.36 ± 1.36	0.042
Interferon β-1b						
DQB1*02:01	5/38	31.50 ± 10.93	63.67 (18-118)	6.25 ± 3.55	4.47 ± 3.13	0.046

DMT: disease modifying therapies; *: Patients with statistical significance; MSSS: Multiple Sclerosis Severity Score; Initial: score at the beginning of treatment; Final: score at the end of treatment; **: Mean (maximum-minimum); ***: Mean ± standard deviation; *T*: Wilcoxon signed-ranks test.

corticosteroids; HLA-DRB1*03:01 (5/5); HLA-DPB1*04:01 (6/7), DQB1*03:02 (7/7) and DQB1*06:02 (7/7), HLA-C*07:02 (5/5) treated with azathioprine; HLA-DRB1*11:04 (6/6), DQB1*03:01 (5/5) and DQB1*03:02 (5/5) treated with interferon β-1a 22 mcg; HLA-DPB1*02:01 (5/5); HLA-C*05:01 (5/5) treated with interferon β-1a 30 mcg; HLA-DQB1*02:01 (5/6) treated with interferon β-1b (Table 4). For the other alleles, there was no statistically significant relationship (Table 4).

DISCUSSION

High-resolution HLA sequencing techniques have been described for some years and have enabled many different HLA alleles to be identified in various populations[21,22]. Because of its multi-ethnic nature, the southern Brazilian population, which was previously classified as white skinned (95.6%) and black skinned from Afro-descendence (3.2%), has a great diversity of HLA alleles as well as HLA profiles different from those of the European populations. In our study, the HLA profile revealed that diversity, for example, the HLA-DRB1*15:01 found more frequently in Europeans, had a frequency of only 14% in MS patients and in 8.1% in controls in south Brazil[10]. Many different alleles were also found in the present study, resulting in fewer patients for each type of treatment (Table 2).

Regardless of the DMT used, the EDSS increased in all the groups in our study when these were analyzed together, and showed a statistically significant relationship with disease progression and time of onset, in agreement with the current literature[1,3]. The EDSS is a useful and widely-used scale to measure the MS progression[17]. Invariably, most patients increased in their disability despite the type of therapy; this being more intense in the first years of the disease due to inflammatory phase activity before their entry into the degenerative phase[3,23]. Most of the studies on DMTs assessing the disability using the EDSS were over a two-year study period. These studies showed variable benefits comparing placebo with other DMTs or corticosteroids, interferon β-1a 44 mcg t.i.d., interferon β-1a 30 mcg IM weekly, mitoxantrone, natalizumab, fingolimod and teriflunomide[24]. There has been insufficient evidence for azathioprine, interferon β-1b 8 MIUs and methotrexate. No benefit was found with the use of glatiramer acetate[24].

We chose to use the MSSS because it relates the EDSS to the distribution of disabilities in patients with comparable disease durations[18]. Analysis of the MSSS for our patients revealed a reduction in disability when all the HLA alleles and the DMTs were analyzed as one group (all patients together). A large proportion of our patients showed a reduction in this score, and this varied according to the allele type in each HLA class. For some specific HLA types, there was a reduction in MSSS when our patients were treated with corticosteroids, azathioprine, interferon β-1a 22 mcg, interferon β-1a 30 mcg and interferon β-1b (Table 4).

Our data found a relationship between the use of a corticosteroid alone over 12 to 278 months, and a decrease on the MSSS in patients with HLA-DRB1*15:01, DPB1*04:01, DQB1*02:01 and DQB1*03:01, suggesting stabilization or improvement of the disability. In relapses, IV corticosteroid therapy has led to a high rate of complete or partial recovery of symptoms in short-term follow-up[25]. However, some patients with specific HLA alleles have shown good response with reduction or slow progression of the disability after several years using only prednisone in multiple "short time" therapies (2-3 weeks), instead of a high dose for a few days[26]. Nevertheless, our review of the literature failed to reveal any study correlating the HLA and prednisone therapy.

The patients with HLA-DRB1*03:01, HLA-DPB1*14:01, DQB1*06:02 and DQB1*03:02 treated with azathioprine showed a reduction on the MSSS ($p = 0.018$ to 0.043) indicating improvement or stabilization of the disability. Despite that azathioprine is not widely used in MS treatment, some studies have shown similar effects of the immunomodulatory drugs for relapses, progression of disability and number of lesions on MRI[27-30]. A study using azathioprine and serological HLA typing showed progression of the disease during treatment in patients with HLA-A1-B8, HLA-B8-DR3 and HLA-A1-B8-DR3, and no difference for HLA-B7 and HLA-DR2[31]. Later, a trial with azathioprine, which included some data on serological HLA typing, revealed a small benefit, as measured by the EDSS, for patients with HLA-DR3 and HLA-DR2 after a three-year period but without a relationship with the HLA subtype[28]. However, all these studies used low resolution techniques to identify the HLA type.

The patients treated with interferon β-1a 22 μg (HLA-DRB1*11:04, DQB1*03:01 and DQB1*03:02), interferon β-1a 30 μg (HLA-DPB1*02:01 and HLA-C*05:01) and interferon β-1b (DQB1*02:01) decreased their MSSS ($p < 0.043$ to $p < 0.027$), suggesting improvement or stabilization of their disability (duration of treatment six to 90 months). In an earlier study that used low-resolution HLA typing techniques, a reduction in MS relapses after one year of treatment with interferon β-1a (22 mcg t.i.w. subcut) was found in patients with HLA-DRB1*03, HLA-DQB1*03 and HLA-DQB1*02[32]. In another study, a beneficial response (reduced disease relapses and stabilization of EDSS scores in the two years of follow-up) was reported with interferon β-1a (20 μg t.i.w., IM) in patients with HLA-DRB1*04 or the HLA-A*03-DRB1*04 haplotype[13].

We did not find any statistical relationship in our patients using glatiramer acetate. However, there is a report that glatiramer acetate and the presence of HLA-DR15 and HLA-DQ6, or absence of HLA-DR17 and HLA-DQ2, prevents relapses and halts the disease progression[33]. Also, the presence of HLA-DRB1*15:01, HLA-DQB2/DOB and HLA-DOB/TAP2, as well as several single nucleotide polymorphisms (SNPs) in

genes involved in the inflammatory cascade, were associated with a reduction in the annual relapse rate within two years[14].

Methotrexate is not widely prescribed for MS at the present time, but although previous studies have shown some efficacy on specific clinical grounds, in spite of its efficacy being inferior to interferon β-1a, methotrexate is used more frequently as add-on therapy[34]. No study was found correlating methotrexate and HLAs in MS patients.

A genome-wide pharmacogenetics investigation found statistically significant differences in response to interferon β therapy between some individuals with different SNPs in a population from France and Spain with a predominant HLA-DRB1*15:01 genotype (38%)[35]. Modest relationships were observed between SNPs from several genes outside the HLA system and response to DMTs in European and American populations. The relationship was found in MS patients who were responders and non-responders to interferons and glatiramer acetate, in some patients with HLA-DRB1*15:01, DQB2 and DOB/TAP2[7,33]. However, the one limitation of these studies was because they did not compare the classic HLA types *versus* SNPs in the other genes[14,36]. These SNPs were different in each study, suggesting that there are factors other than the genetic component mediating the response to immunomodulators[8,35,37,38].

In our study, the fact that there were many different HLA types "diluted" the number of patients for each allele, making it difficult to carry out a statistical analysis in our population who had allele distributions different from Europeans[10]. Ours is a report of a real-world outpatient clinic, with the inclusion of patients with six months of treatment, showing that most of the DMTs had an effect on the disability in the long term, in the majority of patients, even without statistical significance (Table 4). This was not an artificial controlled trial study, where the patients who did not fit the protocol were excluded. The patients with six months of treatment changed to a different DMT because they had collateral effects, allergy, severe depression, more than one severe exacerbation, increased their EDSS by several points or developed new lesions on magnetic resonance imaging.

We failed to find similar studies in the literature that compared HLA subtypes using high-resolution HLA typing techniques and correlated the HLA type with the disability score, disease duration and different forms of therapy. In addition, the HLA profiles in the populations of previous studies were different from those of our population and the interplay of the innumerable genes outside the MHC but present in several sub-sets of cells involved in the pathogenesis of MS, may interfere with the results[39,40]. Also, there is growing evidence of external factors—such as UV radiation, vitamin D, gut microbiomes, viral infections, smoke, sodium intake and others—may have some influence on the pathogenesis of MS and may be more important than the immune genetic profile of the patient in the efficacy of DMTs[41-44].

In conclusion, our study showed a relationship between the HLA and the effect of DMTs on some HLA class I and II alleles in some patients. We observed a decrease in the MSSS for certain HLA genotypes, which might reflect a better response to different DMTs in a few patients. These results should be interpreted with caution because of the small number of patients with some types of HLA and DMTs.

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