

# Genetic variant HLA DRB1\*0403 and therapeutic response to diseases modifying therapies in Multiple Sclerosis: A case-control study.

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## Article

# Genetic Variant HLA DRB1\*0403 and Therapeutic Response to Diseases Modifying Therapies in Multiple Sclerosis: A Case-Control Study

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**Abstract:** Multiple sclerosis (MS) is a chronic and demyelinating disease with an autoimmune origin, which lead to neurodegeneration and progressive disability. Approximately 30 to 50% of patients do not respond optimally to Disease Modifying Therapies (DMTs), therapeutic response may be influenced by genetic factors such as genetic variants. Therefore, our study aimed to investigate the association of HLA DRB1\*0403 genetic variant and therapeutic response to DMTs in MS. We included 105 patients with MS diagnosis. No evidence of disease activity based on absence of clinical relapse, disability progression or radiological activity (NEDA-3), was used to classify therapeutic response. Patients were classified as follows: a) controls: patients who achieved NEDA-3; b) cases: patients who did not achieve NEDA-3. DNA was extracted from peripheral blood leukocytes. HLA DRB1\*0403 genetic variant was analyzed by quantitative polymerase chain reaction (qPCR) using TaqMan probes. NEDA-3 was achieved in 86.7% of MS patients treated with DMTs. Genotype frequencies were: GG 50.5%, GA 34.3% and AA 15.2%. No differences were observed in the genetic variant AA between patients who achieved NEDA-3 versus patients who did not achieve NEDA-3 (48.7% vs. 43.1%,  $p=0.6$ ). We concluded that in Mexican patients with MS, HLA DRB1\*0403 was not associated with therapeutic response to DMTs.

**Keywords:** multiple sclerosis; therapeutic response; HLA DRB1\*0403

1. Introduction

Multiple Sclerosis (MS) is the most frequent demyelinating inflammatory disease of the central nervous system. MS is the second cause of disability in young adults [1,2]. Worldwide MS prevalence ranges from 5.5 to 29.5%, the higher prevalence has been observed in North America, Western Europe and less frequently in countries near Ecuador [3]. Precise etiology of MS is not yet well understood, but genetic, geographical, and environmental factors have been described [4]. Major histocompatibility complex (MHC) is the key susceptibility locus in MS. MHC class II appears to have a stronger association with MS than class I. About 200 polymorphisms have been studied for the susceptibility of developing MS [5]. This association has been fine-mapped to the extended haplotype HLA-DRB1 was the most correlative. In a meta-analysis, Zhang et al., found that DRB1\*03 and DRB1\*04 phenotypes are significantly associated with disease state [6], and these alleles are related to a worse prognosis when considering the time taken to reach severe disability [7] DRB1\*04 is the most prevalent DRB1 allele group in the Mexican population [8].

Over the past 50 years, the MHC locus has been shown to influence many critical biological traits and individual susceptibility to autoimmune diseases [9]. Studies have also evaluated HLA-DRB1 haplotypes in other pathologies, demonstrating that HLA-DRB1 haplotypes are associated with radiological severity, mortality, and response to TNF- $\alpha$  inhibitor drugs [10].

The current management strategies focus on treating acute attacks, ameliorating symptoms, and reducing biological activity through disease-modifying therapies (DMTs) [11]. These pharmacological treatments modify the course of MS through suppression or modulation of immune function, preventing the transition of autoreactive T and B lymphocytes across the blood-CSF barrier and through autoreactive lymphocyte depletion [12,13]. The efficacy of different DMTs has been demonstrated in various studies. However, approximately 30 to 50% of patients do not respond optimally to DMTs, showing any response in some cases [14,15]. Early prediction of the response to treatment continues to be one of the main difficulties in treating patients with MS [16] Multiple factors related to an adequate therapeutic response are unknown [17]. Therefore, it is necessary to explore the response to treatment and its relationship with the genes to personalized medicine in MS. The present study investigates the potential association of the genetic variant HLA DRB1\*0403 and therapeutic response to DMTs in MS.

2. Results

Genotype distributions of this polymorphism were consistent with the Hardy-Weinberg equilibrium ( $p>0.5$ ). Table 1 presents the characteristics of 105 patients with MS who were assessed. Patients with MS had a mean age of  $38.9 \pm 10.2$  years, and it was more frequently observed in the female sex. The mean disease duration was  $8.8 \pm 5.8$  years. No disease activity was achieved in 86.7 % of MS patients. About the genetic characteristics, wild homozygote was observed in 50.5% of MS patients.

Table 1. Selected characteristics in Multiple Sclerosis patients.

Variables	Multiple Sclerosis n = 105
Age (years), mean $\pm$ SD	38.9 $\pm$ 10.2
Female, n (%)	70 (66.7)
<b>Disease characteristics</b>	
Disease duration (years), mean $\pm$ SD	8.8 $\pm$ 5.8
EDSS score, mean $\pm$ SD	2.9 $\pm$ 1.9
EDSS $\leq$ 4, n (%)	75 (71.4)
EDSS $>$ 4, n (%)	30 (28.6)505.5%
NEDA-3 achieved, n (%)	91 (86.7)
NEDA-3 not achieved, n (%)	14 (13.3)
<b>Disease-modifying therapies</b>	

Glatiramer acetate, mg / mL, n (%)	39 (37.1)
Interferon beta, UI, n (%)	30 (28.6)
Rituximab, mg, n (%)	9 (8.6)
Fingolimod, mg, n (%)	14 (13.3)
Azathioprine, mg, n (%)	5 (4.8)
Natalizumab, mg/ mL, n (%)	3 (2.9)
Dimethyl fumarate, mg, n (%)	5 (4.8)
<b>Genetic Variant HLA DRB1*0403</b>	
<i>Genotype</i>	
G/G, n (%)	53 (50.5)
G/A, n (%)	36 (34.3)
A/A, n (%)	16 (15.2)
<i>Allele</i>	
G, 2n = 142 (%)	142 (67.6)
A, 2n = 68 (%)	68 (32.4)

Quantitative variables are expressed as mean and  $\pm$  standard deviation SD; Qualitative variables are expressed in frequency and percentage (%); EDSS: Extended Disability Scale; NEDA: No evidence of activity disease.

Table 2 shows the comparison between MS patients who did not achieve NEDA-3 versus MS patients who achieved NEDA-3. We found a higher frequency of glatiramer acetate use in MS who achieved NEDA-3. No differences were observed in age ( $37.7 \pm 10.1$  vs.  $39.1 \pm 10.3$ ,  $p=0.6$ ), disease duration ( $8.8 \pm 5.4$  vs.  $8.8 \pm 5.9$ ,  $p=0.9$ ), and the different treatments.

**Table 2.** Comparison of clinical variables between the case group (NEDA-3 not achieved) and control group (NEDA-3 achieved).

	NEDA-3 not achieved (Case group) n=14	NEDA-3 achieved (Control group) n=91	<i>p</i>
Female, n (%)	12 (85.7)	59 (53.7)	0.1
Age (years), mean $\pm$ SD	$37.7 \pm 10.1$	$39.1 \pm 10.3$	0.6
EDSS score, mean $\pm$ SD	$3.6 \pm 2.3$	$2.8 \pm 1.9$	0.2
Disease duration (years), mean $\pm$ SD	$8.8 \pm 5.4$	$8.8 \pm 5.9$	0.9
<b>Disease-modifying therapies</b>			
Glatiramer acetate, n (%)	2 (14.3)	37 (40.6)	0.05
Dimethyl fumarate, n (%)	1 (7.1)	4 (4.4)	0.5
Fingolimod, n (%)	3 (21.4)	11 (12.1)	0.3
Interferon beta, n (%)	4 (28.6)	26 (28.6)	1.0
Natalizumab, n (%)	1 (7.1)	2 (2.2)	0.3
Rituximab, n (%)	2 (14.3)	7 (7.7)	0.4
Azathioprine, n (%)	1 (7.1)	4 (4.4)	0.7

Quantitative variables are expressed as mean and  $\pm$  standard deviation SD; Qualitative variables are expressed in frequency and percentage (%); EDSS: Extended Disability Scale; NEDA: No evidence of activity disease.

Table 3 compares genotypes and allele frequencies of genetic variant HLA DRB1\*0403 between MS patients who achieved NEDA-3 and those who did not achieve NEDA-3. There were no significant differences between the frequency of genotypes in the comparison between MS patients who achieved NEDA-3 and those who did not achieve NEDA-3. Additionally, in the genetic models, no differences were observed when carriers of wild homozygote, heterozygote, or mutated homozygous for achieve NEDA-3, indicating no association between genetic variant HLA DRB1\*0403 and therapeutic response to disease-modifying therapies in MS patients.

**Table 3.** HLADRB1 \*0403 genetic variant as a predictor therapeutic response in patients with Multiple Sclerosis treated with disease-modifying therapies .

Multiple sclerosis (n=81)	NEDA-3 not achieved (Case group) n=14	NEDA-3 achieved (Control group) n=91	OR	95%CI	<i>p</i>
<i>Genotypes</i>					
GG, n= 53 (%)	8 (57.1)	45 (49.5)	-	-	0.6
GA, n=26 (%)	5 (35.7)	31 (34.1)	-	-	
AA n= 3 (%)	1 (7.1)	15 (16.5)	-	-	
<i>Genetic models</i>					
Dominant Model (GG vs. GA + AA)	-	-	0.73	0.23-2.28	0.80
Recessive Model (GG+GA vs. AA)	-	-	1.39	0.43-3.21	0.6
<i>Alleles, 2n= 162</i>	2n =28	2n=182			
G allele, 2n = 141 (%)	21 (75)	121 (66.5)		Referent	
A allele, 2 n= 68 (%)	7 (25)	61 (33.5)	1.51	0.61-3.75	0.36

No evidence of activity disease; GG: wild homozygote; GA: heterozygote; AA: mutated homozygous; OR: odds ratio risk; 95% CI: 95% confidence interval; p value .

### 3. Discussion

The present study analyzes the potential association of the genetic variant HLA DRB1\*0403 and therapeutic response to DMTs in MS. HLADRB1\*0403 was not associated with therapeutic response to DMTs in MS. Wild homozygote (GG) was the most frequently observed in the Mexican Mestizo population with MS. Our results indicate that the frequency of the HLA DRB1\*0403 (A allele) was 32.4% in MS patients from Western Mexico, which is higher than that observed in people with MS in the center of Mexico (10.78%) [18].

To date, to the best of our knowledge, this is the first study to observe the lack of association between this genetic variant and therapeutic response in MS patients. There are no studies in Mexico that report the results of the presence of this or another genetic variant in the mestizo population in Mexico and the response to treatment with respect to DMTs in MS. The risk of non-response to DMTs was similar in the different disease-modifying therapies.

We studied this HLADRB1\*0403 genetic variant as it is the most prevalent in our population in western Mexico. As we know, this HLADRB1 can promote immunogenic presentation share valine (V) at position 86 of the DRB1 groove, which may modulate peptide anchoring and, therefore, the way the neurogenic peptide presents to the TCR of those Th1 cells involved in myelin essential protein (MBP) destruction as reported in other studies. HLA-DRB1\*04 alleles (HLA-DRB1\*0401 and \*0408) by a glycine-to-valine substitution in position 86 of the epitope-binding alpha-helix of the HLA class II molecule, the peptide-binding motif of might promote binding and presentation of an immunogenic peptide and eventually break T cell tolerance and facilitate antibody development to INF $\beta$  [19].

Previous studies have evaluated the frequency of HLA DRB1\*0403, observing an association between the genetic variant and the risk of developing multiple sclerosis in different populations [18-21] In a meta-analysis Zhang et al., reported the heterogeneity of DRB1\*04 frequencies were too high to merge data. [22] Therefore, DRB1\*04 cannot be a good predictor or MS in Caucasians However, the heterogeneity was markedly reduced by analyzing 4-digit genotypes of DRB1\*04 separately. Consequently, further studies should focus on 4-digit genotypes of DRB1\*04.

On the other hand, similar to our study, it has been reported in MS patients the association of HLA DRB1\*0403 with a non-therapeutic response. Buck et al., reported in a post hoc analysis in 941 patients treated with interferon  $\beta$ -1b an increased risk for carriers of HLADRB1\*04:01 (OR=3.3) and carriers of HLA-DRB1\*07:01 (OR=1.8) for developing neutralizing antibodies to INF $\beta$  [23]. Moreover, Hoffmann et al., found an association between HLADRB1\*0401 and HLA-DRB1\*0408 positive patients and the development of antibodies to INF $\beta$  [19]. INF $\beta$  exhibits immunogenicity like other



protein-based disease-modifying agents; up to 50% of patients may develop antibodies to INF $\beta$ , of which a significant proportion neutralize the activity of INF $\beta$  [24, 25]. The development of antibodies to INF $\beta$  has been considered a significant factor contributing to clinical treatment failure. Our results differ from the observation of other populations. Mazdeh et al., reported a beneficial response, analyzed by reduced disease relapses and stabilization of EDSS scores in the two years of follow-up, with interferon  $\beta$ -1a in patients with HLA-DRB1\*04 and HLA-A\*03-DRB1\*04 haplotype [26]. Furthermore, Romero Pinel et al., reported that HLA-DRB1\*04 allele was associated with a worse prognosis when considering the time taken to reach severe disability [7].

Our study observed an adequate response to DMTs in 86.7% of the patients, which supports new studies in search of other genetic variants that may be associated with an adequate response to treatments. NEDA-3 was achieved in 33.6% of the patients with MS. NEDA-3 was achieved in 30.9% of patients receiving INF $\beta$ , which agrees with the literature published in the West [27]. In another study where NEDA was defined as 12-week confirmed disability progression, no protocol-defined relapses, no new/enlarging T2 lesions, and no T1 gadolinium-enhancing lesions, the results indicated NEDA was increased in MS patients treated with ocrelizumab vs. MS patients treated with IFN $\beta$ -1a (66.4% vs. 24.3%,  $p < 0.001$ ) [28].

There are some strengths in our study. The most important is that we used a stricter definition to evaluate therapeutic response DMTs that are according to the current concepts and goals of treatment in MS. Another strength of our study is that we analyzed the therapeutic response with different available clinical practice DMTs.

However, our study has some limitations that must be considered. Other polymorphic sites could be implicated in the development therapeutic response to DMTs in MS. Further studies should include genetic expressions associated with these genotypes as well as the influence from other factors, such as interactions between diverse genetic variations. Linkage disequilibrium within other genetic variants should be analyzed. It has been suggested that the presence of a specific haplotype in the homologous chromosome could have an additive effect on the genetic susceptibility to response in MS.

Future investigations that could replicate the findings in this work are necessary to verify the biological precept of the plausibility of gene-environment interactions with therapeutic response to DMTs in MS. This study might reflect only the genetic characteristics of patients with OP from the Western population of Mexico. Therefore, a multicenter study including patients from other regions of Mexico should be performed. On the other hand, although these multicenter studies are required, they probably would not modify our main conclusion that this variant has no significant influence.

## 4. Materials and Methods

### 4.1. Study Design

Case-control study

### 4.2. Study Population

This study included 105 patients with Multiple Sclerosis recruited from an outpatient clinic of West Medical Center in Guadalajara, Mexico, who were enrolled from January 24, 2019, through February 28, 2020. Inclusion criteria were the following: 1) aged  $\geq 18$  years, 2) Diagnosis of MS according to the 2017 criteria of McDonald [29], 2) Mexican Mestizo defined according to the Mexican National Institute of Anthropology and History (INAH) as: "individuals who were born in Mexico, of the 3rd generation including their own and who were descendants of the original autochthonous inhabitants of the region and individuals who were mainly Spaniards" [30], 3) treatment with DMTs, and Brain Magnetic Resonance Image (MRI). To ensure a correct diagnosis, a neurologist performed the diagnosis; we performed 1.5-tesla MRI with conventional T1, gadolinium-enhanced T1, T2, and fluid-attenuated inversion recovery (FLAIR) sequences and confirmed the presence of typical round hyperintense lesions in T2 and FLAIR sequences and hypointensity in T1 with or without enhancement distributed in a different location; the time of evolution differed for each patient [31].

Oligoclonal bands were not considered mandatory to support or exclude an MS diagnosis G [32]. Patients with kidney or liver disease diagnoses and other uncontrolled autoimmune or psychiatric diseases and pregnancy were excluded.

#### 4.3. Clinical Setting

This study included patients with MS referred from a tertiary-care center (UMAE, Hospital de Especialidades, Centro Médico Nacional de Occidente [CMNO]) in Guadalajara, Mexico

#### 4.4. Clinical Assessments

All patients were assessed for clinical and neurological evaluation. Disease activity was assessed using the outcome No Evidence of Disease Activity (NEDA-3), which was achieved when MS patients present any of the composites. The first composite of NEDA-3 was the absence of clinical relapse. Relapse was defined as a single acute or subacute episode of focal neurologic symptoms, informed by the patients in at least 24 hours, with or without recovery, in the lack of fever or infection. The second composite was absence of disability progression. Functional systems were used to establish the disability score according to Kurtzke's Extended Disability Status Scale (EDSS). Progression was defined as an EDSS increase of 1.5 or more if the baseline was zero, by an increase of one if the baseline was less than 5.5, and by an increase of 0.5 if the baseline was 5.5 or more. EDSS considers visual, brainstem, pyramidal, cerebellar, sensory, bowel and bladder, cerebral functions and ambulation. EDSS minimum score obtained is equal to 0 points and the maximum score, equal to 10 points. MS patients were classified with relative severe dysfunction when the score was  $\geq 4$  points [33]. The third composite was the absence of radiological activity. Active lesions were evaluated by magnetic resonance image (MRI). Radiological activity was defined as the occurrence of contrast-enhancing lesions on T1-weighted or new/enlarging hyperintense lesions on T2-weighted brain or spinal cord [31].

NEDA-3 was achieved when MS patients were presented with no relapses, no disability progression, and no radiological activity (Control group). Therefore, treatment failure (Case group) was defined as, within the last year of treatment, presenting any of the following composites: a clinical relapse or disability progression or radiological activity.

#### 4.5. Genotyping

Genomic DNA from 105 subjects was extracted from peripheral blood leukocyte samples using the modified Miller technique [34]. Genomic DNA was quantified using a Nanodrop Genomic, and the DNA was diluted in Tris-EDTA buffer to 20 ng/ $\mu$ L and placed in 200  $\mu$ L propylene cryotubes (Eppendorf<sup>TM</sup>). Genotyping of HLA DRB1\*0403 polymorphism was performed by quantitative polymerase chain reaction (qPCR) using TaqMan probes [35]. TaqMan Assay IDs C\_2958435\_10 was performed according to the manufacturer's instructions (Applied Biosystems); the StepOne<sup>TM</sup>, Real-Time polymerase chain reaction (qPCR) system was employed for this purpose (Applied Biosystems). All results were independently analyzed by two investigators blinded to patient information. In case of ambiguous results, the sample was examined a second time. The resulting genotypes for the genetic variants were classified into one of the following three categories: wild homozygote (GG), mutated homozygous (AA), and heterozygote (GA). In the present study, we adopted the following genetic models: dominant (GG vs GA + AA) and recessive (GA+AA vs GG).

#### 4.6. Statistical Analysis

Qualitative variables were expressed as frequencies (%), while quantitative variables as means and standard deviation (SD). We identified genotype frequencies by direct counting. Allele frequencies were determined by counting from the observed genotype frequencies. Comparisons between means were computed using the independent sample Student *t*-test. Comparisons between proportions were carried out using the chi-square test (or the Fisher exact test if required). Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated.

## 5. Conclusions

In conclusion, the HLADRB1\*0403 genetic variant does not confer a risk to achieve therapeutic response with DMTs in Mexican mestizo patients with MS

The search for other genetic variants explaining the therapeutic response in MS patients treated with DMTs continues to be ongoing.

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**Informed Consent Statement:** Informed consent was obtained from all patients involved in the study.

**Data Availability Statement:** The data are not publicly available due to reasons of sensitivity. The data presented in this study are available on request from the corresponding author.

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