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Presence of the HLADR13 allele among Mexican Mestizos suggests a protective factor against relapsing-remitting multiple sclerosis (RRMS)

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ABSTRACT

Background and objective: Multiple sclerosis (MS) is a chronic demyelinating disease that affects the central nervous system. Researchers have looked for an association between relapsing-remitting MS (RRMS) and human leukocyte antigen (HLA) as risk or protective factor associated to ethnicity, which may add a partial explanation to disease heterogeneity and geographical variations. We described the frequency of the HLA-DR alleles in Mexican Mestizo (RRMS) patients.
Patients and methods: We included 143 RRMS patients and 377 healthy controls, both Mexican Mestizos. Previous signing informed consent, we record demographic and clinical characteristics of the participants. Genetic profile was made, and HLA frequencies in both groups were compared.
Results: RRMS patients were 39.8% male and 60.2% female, mean age was 35 years. While, controls were 48%male and 52% women, mean age was 38 years. The most frequent allele found in subjects with RRMS was DR 15 ($p = 0.006$, OR = 2.2, 95% CI: 1.3–3.6). DR 13 allele was more frequent among healthy subjects than RRMS patients ($p = 0.050$) with a protective OR 2.6, (95% CI: 1.3–5.2, $p = 0.050$).
Conclusion: In our study we found HLA DR 13 was more frequent in healthy controls than in RRMS patients, suggesting a protective factor among Mexican Mestizo population

1. Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease that affects the central nervous system and is one of the most disabling neurological diseases among young people [1,2]. There are approximately 2.3 million of MS patients in the world [3]. Relapsing-remitting MS (RRMS) is more frequent in Caucasians, especially in the northern and southern latitudes as North America, Europe and Australia [3,4]. Recent studies document that MS frequency is increasing in Latin America. In Mexico, the prevalence of MS is low, but it has increased from 1.6 to 12 patients per 100,000 inhabitants in the last years [5–7]. Both, environmental and genetic factors are involved in the development of MS [2,4]. Several genes have been studied, often with mixed results and the only definitive association to MS is with the human leukocyte antigen HLA-DR2 (DRB1*1501, DQB1*0602) haplotype on chromosome 6p21 [8–12]. HLA-DR is a major histocompatibility complex (MHC) class II cell surface receptor, which primary function is to present peptide antigens to the immune system for regulation of T-(helper)-cell responses. Researchers have looked for an association between HLA genes as risk or protective factor for MS, but the results have been inconsistent. The ethnic variability could explains the HLA alleles heterogeneity of MS and the observed geographical variations [13]. The degree of association between MS and HLA genes vary considerably among populations [14–18]. In MS Brazilian patients, allele HLA-DRB1* 11 was reduced compared to controls, suggesting a protective factor [19]. Mexican population is composed of Mestizos, term used to describe people with a mixture of European and indigenous descent, and non-mixed American Indian groups [6,20,21]. One of this groups named Lacandonians have not MS disease [22]. Otherwise, Ordoñez and collaborators showed that

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Table 1
RRMS patients clinical characteristics.

	Male $n = 57$	Female $n = 86$	Total = 143	p value
Age in years, mean (±SD)	34.5 (±8.5)	35 (±9.1)	35 (±9.5)	0.89
Time of disease evolution (years)	4.0	7.0	6.0	0.00
EDSS	4.5	4.5	4.5	1.84

RRMS = Relapsing remitting multiple sclerosis, SD = standard deviation, EDSS = Expanded Disability Status Scale.

Table 2
Genes frequency of HLA-DR alleles in RRMS patients compared with normal Mexican Mestizo.

HLA-DR	Cases N:143		Controls N:377		pC*	OR	CI:95%
Alleles	286	gf	754	gf			
DR 04	72	0.252	195	0.257			
DR 08	52	0.182	93	0.122			
DR 15	36	0.126	45	0.059	0.006	2.2	1.3–3.6
DR 16	25	0.087	38	0.049			
DR 01	21	0.073	62	0.081			
DR 03	21	0.073	55	0.071			
DR 07	16	0.056	69	0.090			
DR 14	17	0.059	67	0.087			
DR 11	13	0.045	46	0.060			
DR 13	10	0.035	66	0.086	0.050	0.3**	0.18–0.77
DR 12	2	0.007	6	0.007			
DR 10	1	0.003	12	0.015			
Total	286	–	754	–			

RRMS = Relapsing remitting multiple sclerosis. gf = frequencies.
Absences values are not significant.
* pC: p value corrected by Bonferroni method.
** OR: Odds Ratio of 0.3 represents a protective factor of 2.6 (95% CI: 1.3–5.2, $p = 0.050$).

Mexican Mestizos patients with MS have different genetic admixture proportions than healthy Mestizos, making them susceptible to developing MS because of genetic European component. This work also showed ASF1B as a potential protective gene for MS in Mexican Mestizo population [23].

The HLA DR13 allele has been studied in the host response to infectious processes such as hepatitis C virus and in the immune response in patients with autoimmune diseases such as systemic lupus erythematosus [24,25]. DR13 has been a controversially associated to MS, because Weinshenker and collaborators found positively association with MS [26]. However, recent investigation associated HLA DR13 with Italian benign MS patients [27].

The aim of our study was to explore the presence of HLA DR in Mexican Mestizo RRMS patients and study its association with disability.

2. Patients and methods

2.1. Patients

RRMS subjects were invited to participate and they were selected from the Laboratory of Clinical Neurodegenerative Diseases of the National Institute of Neurology and Neurosurgery, in Mexico City. Our sample included 143 RRMS patients according to McDonald criteria [28], recruited following a non-probability sampling rate in a 10-month consecutive period. Patients and controls underwent genetic profile and patients were followed-up at the outpatient clinic. Progression of MS was defined as progression index (PI) calculated by dividing the Expanded Disability Status Scale (EDSS) by the number of years of disease course. For our study we included patients with a PI of >0.61 (expect or normal progression in RRMS patient is 0.4–0.6). We included 377 healthy controls. Patients and controls were Mestizos recruited from the same geographical area of Mexico City and its surrounding metropolitan area.

2.2. Samples processing

After signing the informed consent formats, we collected a 10 mL blood sample from all consenting patients at the time of recruitment. Samples were processed using standard procedures at the clinical laboratory of the National Institute of Medical Sciences and Nutrition at Mexico City. Plasma was separated from blood at the laboratory together with buffy coat and erythrocytes and stored at −70 °C until it was needed to analyze. Biological waste was managed in accordance with international standards both at the hospital where the samples were taken and at the laboratory where they were processed. We extracted DNA from whole blood samples from 3 ml leukocytes sediment (pellet) collected with EDTA as an anticoagulant. DNA was extracted using the ABI PRISM™ semi-automated equipment Nucleic Acid PrepStation 6100. DNA purity was assessed with spectrophotometry with the association of the A260/A280 readings, which should be between 1.65 and 2.0 [29]. We ran 0.8% agarose gels and assessed DNA integrity. Genotyping was performed using PCR-SSCP and PCR-RFLP. The genetic frequency of the HLA allele was obtained by direct counting.

2.3. Statistical methods

We calculated the mean, median and standard deviation (±SD) of continuous variables. Categorical variables were expressed as proportions, and comparisons were performed with a chi-square test. For variables with normal distribution, a t-test was performed for two means and chi square test in abnormal distribution variable. Also, Bonferrioni correction for multiple comparisons was made for to avoid error type I. Pearson test was used for correlation analysis. We used SPSS software for Windows, (V.16.0, IBM Inc., Armonk, New York, USA) for statistical analyses. A p value of less than 0.05 was deemed as statistically significant.