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HLA-DRB1 alleles and genotypes in patients with relapsing-remitting multiple sclerosis from western Mexico: a pilot study

José Alfonso Cruz-Ramos[1], Genaro Gabriel Ortiz[1], Eduardo Ignacio Díaz-Barba[1], Miguel Angel Macías-Islas[2] and Mario Mireles-Ramírez[2].

[1]Laboratorio de Desarrollo-Envejecimiento; Enfermedades Neurodegenerativas; División de Neurociencias. Centro de Investigación Biomédica de Occidente (CIBO). Instituto Mexicano del Seguro Social (IMSS). Guadalajara, Jalisco. México.
[2]Departamento de Neurología. Unidad Médica de Alta Especialidad (UMAE)- Hospital de Especialidades, Centro Médico Nacional de Occidente (CMNO). IMSS. Guadalajara, Jalisco. México.

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Abstract. The *HLA-DRB1* alleles are closely related to genetic susceptibility to multiple sclerosis (MS). There is a high prevalence of MS in Caucasian populations, whereas non-Caucasian populations have a low prevalence. In Latin American countries, genomes are made up of a heterogeneous gene pool, which varies between different regions of each country. The objective of this study was to evaluate the association of alleles and genotypes of *HLA-DRB1* in relapsing-remitting multiple sclerosis (RRMS) in Mexican Mestizos of western Mexico. *HLA-DRB1* alleles and genotypes were determined for 59 RRMS patients and 434 healthy subjects. The results show that allele *HLA-DRB1**15 (p: 0.013, OR: 2.74) was associated with risk of RRMS, no others alleles were significantly associated. Whereas, a risk tendency was observed for alleles *HLA-DRB1**09 (p: 0.062, OR: 6.24), *HLA-DRB1**04 (p: 0.086, OR: 1.876) and genotypes *HLA-DRB1**15/*13 (p: 0.068, OR: 15.71), *HLA-DRB1**15/*04 (p: 0.068, OR: 6.35). Otherwise, the alleles *HLA-DRB1**08 (p: 0.062, OR: 0.340) and *HLA-DRB1**01(p: 0.062, OR: 0.29) show tendency to a protective effect. Further research is required about risk alleles in larger patient RRMS cohorts in western Mexico. *HLA-DRB1* risk allelic variation in different regions of Mexico for MS further illustrates the genetic heterogeneity of the Mexican population and the Caucasian influence in shaping the epidemiology of MS in Mexico.

Corresponding author: Genaro G. Ortiz. Laboratorio de Desarrollo-Envejecimiento; Enfermedades Neurodegenerativas. CIBO-IMSS. Sierra Mojada 800 C.P.: 44340. Guadalajara, Jalisco. México. E-mail: genarogabriel@yahoo.com

Alelos y genotipos *HLA-DRB1* en pacientes con esclerosis múltiple remitente-recurrente del occidente de México: un estudio piloto

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Palabras clave: *HLA-DRB1*; esclerosis múltiple remitente-recurrente; alelos; genotipos; riesgo; mestizos mexicanos.

Resumen. Los alelos *HLA-DRB1* están estrechamente relacionados con la susceptibilidad genética a la esclerosis múltiple (EM). Hay una alta prevalencia de EM en las poblaciones caucásicas, mientras que las poblaciones no caucásicas tienen una prevalencia baja. En los países latinoamericanos, los genomas se componen de un grupo genético heterogéneo, que varía entre las diferentes regiones de cada país. El objetivo del estudio fue evaluar la asociación de alelos y genotipos de *HLA-DRB1* en esclerosis múltiple recurrente-remitente (EMRR) en mestizos mexicanos del occidente de México. Se realizó un estudio de casos y controles, se determinaron alelos y genotipos de *HLA-DRB1* de 59 pacientes con EMRR y 434 sujetos sanos. Los resultados mostraron que el alelo *HLA-DRB1**15 (p: 0,013, OR: 2,74) se asoció con riesgo de EMRR, ningún otro alelo se asoció significativamente. Mientras que se observó una tendencia de riesgo para los alelos *HLA-DRB1**09 (p: 0,062, OR: 6,24), *HLA-DRB1**04 (p: 0,086, OR: 1,876) y los genotipos *HLA-DRB1**15 /*13 (p: 0,068, OR: 15,71), *HLA-DRB1**15 /*04 (p: 0,068, OR: 6,35). Además, los alelos *HLA-DRB1**08 (p: 0,062, OR: 0,34) y *HLA-DRB1**01 (p: 0,062, OR: 0,29) muestran tendencia de efecto protector. Se requieren investigaciones adicionales sobre los alelos de riesgo en cohortes de mayor tamaño de pacientes con EMRR en el occidente de México. La variación alélica del riesgo *HLA-DRB1* en diferentes regiones de México para EM ilustra la heterogeneidad genética de la población mexicana y la influencia caucásica en la conformación de la epidemiología de la EM en México.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune inflammatory demyelinating disease of the central nervous system (CNS) that typically affects genetically susceptible young adults (1). This disease involves cognitive and motor impairment, usually has two developmental stages (one inflammatory and another degenerative), mostly occurs around the second or third decade of life; and, has a female:male ratio of 2:1(2,3). Currently, three clinical presentations are recognized: one characterized by episodes of remission and recurrence (remitting-relapsing), another with gradual progression (primary progressive) and third, the conversión of remitting-relapsing to progressive form (secondary progressive). The first two forms account for approximately 85% and 15% of the cases respectively, with conversion of 65% of cases with remitting-relapsing to secondary progressive subtype (4). Although Mexico is considered a country with a low prevalence of MS, recent studies suggest that inhabitants of Mexico, Ar-

gentina, and Brazil actually have an average risk for developing the disease (5). Since 1998, MS is a leading cause of neurological consultation in Mexico, exhibiting the highest incidence in the northern and western regions of the country (6,7).

Several studies have associated certain genes encoding neuronal and immunological proteins with the disease. Particularly, the Major Histocompatibility Complex (MHC) seems to modulate the risk for MS in most populations and may even exhibit an odds ratio (OR) of 5.4 (3,8). Among the MHC genes, some *HLA-DRB1* alleles such as *HLA-DRB1**15, *HLA-DRB1**03, and *HLA-DRB1**04 emerge as the main risk-conferring genetic factors (9–11). In contrast, *HLA-DRB1**01 appears to bestow a protective effect against MS (10,12,13). It has also been reported that patients with MS who carry the *HLA-DRB1**15 allele have an early onset and rapid progression of the disease as well as a favorable response to treatment with glatiramer acetate (14,15). On the contrary, *HLA-DRB1**04 carriers show an unfavorable response to treatment with interferon beta (neutralizing antibody production) and slow disease progression (16). Noticeably, the risk conferred by *HLA-DRB1* alleles depends on the individual's genotype with certain allelic combinations increasing (two- or three-fold) and others decreasing the OR values (10,17).

In accordance with Mexico's history, the genetic pool of the current Mestizo population comprises three main fractions, namely Caucasian, Native American, and African genes. This ancestry varies around the country: there are more Caucasian genes towards the north and more Native American genes to the southeast, whereas the proportion of African genes remains relatively constant (18,19). In Mexico City, it has been reported that *HLA-DRB1**03, *HLA-DRB1**04, and *HLA-DRB1**08 pose risk for MS (11,20), whereas, the phenotype HLA-DR13 (*HLA-DRB1**13/X) appears to protect against MS (21). In unselected Mestizos from the greater Valley of Mexico region, the frequency of the*HLA-DRB1* alleles with risk for MS is: *HLA-DRB1**15 in 5.5-10.5%; *HLA-DRB1**08 in 7.8-16.2%; and *HLA-DRB1**04 in 1.5-4.5% (22). In the present work, we compare *HLA-DRB1** alleles and genotypes between MS patients and healthy subjects from western Mexico.

MATERIALS AND METHODS

The study protocol and informed consent were reviewed and approved by the Ethics and Research Committee of the Social Security Institute of Mexico (Registration/Approval number R-2010-1301-8). All individuals included in the study were invited to participate voluntarily, and they were informed in detail about the research and written consent.

a) Sample Collection. The study population was comprised of 59 unrelated relapsing-remitting multiple sclerosis (RRMS) patients diagnosed according to the revised McDonald criteria, from the Multiple Sclerosis Clinic and 434 healthy unrelated control subjects from the Medical Transplant Unit at the National Western Medical Center of the Mexican Social Security Institute (CMNO-IMSS) in Guadalajara, Jalisco. All individuals were Mexican Mestizos with parents and grandparents from the State of Jalisco (Table I). The patient group had an average age of 36.26 years (± 9.84 years), a mean value of 2.48 points (± 2 points) in the expanded disability status scale (EDSS), and a majority (67.79%) were females. We selected RRMS patients because other forms of MS probably have different HLA allele profiles (23). The healthy controls have an average age of 36.52 years (± 10.51 years) and roughly 1:1 sex ratio (females 53.45%). (Table I).

TABLE I
DEMOGRAPHIC CHARACTERISTICS OF RRMS PATIENTS AND HEALTHY CONTROLS FROM GUADALAJARA, MEXICO.

	RRMS patients (n= 59)	Healthy controls (n= 434)	*p*
Gender			0.0377
Female (%)	40 (67.79)	232 (53.45)	
Male (%)	19 (32.20)	202 (46.54)	
Female:male ratio	2.10	1.14	
Age (years) mean ± SD	36.26±9.84	36.52±10.51	

RRMS: relapsing-remitting multiple sclerosis.
p: chi-square.

b) DNA extraction and typing of *HLA-DRB1*. Five milliliters (in duplicate) of venous blood, collected into tubes containing EDTA, were taken by venipuncture from each of the studied subjects. We used the DNA Isolation Kit for Blood/Bone Marrow/Tissue (Roche Molecular Diagnostics, Meylan, France) to extract DNA, and the specific single-stranded oligonucleotide probe (SSO) technique with the Dynal RELI SSO (Invitrogen, Wirral, United Kingdom) HLA-DRB typing kit for allele typification in all samples. DNA amplification was done using a thermal cycler and biotinylated primers under the following PCR settings: 35 cycles of denaturation at 95 degrees for 15 seconds, annealing at 60 degrees for 45 seconds, and extension at 72 degrees for 15 seconds. Then, the amplicons were denatured to form single stranded DNA and hybridized to nylon SSO-fixed membranes containing the complementary sequences. Binding detection was by colorimetric reaction and the interpretation by Dynal RELI SSO pattern-recognizing software.

Statistical Analysis

HLA-DRB1 alleles and genotypes were counted in both groups. Odds Ratio (OR) values were calculated using the SPSS IBM software, compared by means of the two-tailed Pearson's Chi-Square or Fisher exact test when expected values in any cell are <5, multiple comparisons correction for adjusted-p values was made with SAS University software using false discovery rate (FDR) method (24) and Bonferroni method. Finally, the Hardy-Weinberg (HW) equilibrium of genotypic frequencies in both groups was determined with the online software GENPOP version 4.2.

RESULTS

a) Analysis of *HLA-DRB1* alleles. The frequency of *HLA-DRB1* alleles in 59 MS patients and 434 healthy controls is shown in Table II, whereas, inter-group statistical comparisons are show in Table III. The allele *HLA-DRB1**15 ($p = 0.013$) was the only one that retained statistical significance after corrections for multiple comparisons with OR of 2.74. The alleles *HLA-DRB1**09 and *HLA-DRB1**04 show tendency to increase the risk for developing RRMS, OR values 6.24 and 1.88 respectively; while, in contrast, *HLA-DRB1**08 and *HLA-DRB1**01 showed a protective effect tendency against MS (OR: 0.34 and 0.29, respectively), but none retain significance after multiple comparisons correction. (Table II and III).

TABLE II
FREQUENCY OF *HLA-DRB1* ALLELES IN RRMS PATIENTS AND HEALTHY CONTROLS

HLA-DRB1 Allele	RRMS patients (n=59)		Healthy controls (n=434)	
	Count	Allele Frequency	Count	Allele Frequency
16	2	0.0339	20	0.0461
15	15	0.2542	48	0.1106
14	11	0.1864	94	0.2166
13	16	0.2712	91	0.2097
12	1	0.0169	1	0.0023
11	4	0.0678	64	0.1475
10	0	0.0000	13	0.0300
09	4	0.0678	5	0.0115
08	5	0.0847	93	0.2143
07	9	0.1525	53	0.1221
04	41	0.6949	238	0.5484
03	6	0.1017	62	0.1429
01	4	0.0678	86	0.1982

RRMS: relapsing-remitting multiple sclerosis.

b) Analysis of *HLA-DRB1* genotypes. Out of 68 different genotypes, 27 (39.7%) were present in RRMS patients, 66 (97.06%) in control subjects, and 25 (36.76%) in both groups. The two groups were in Hardy-Weinberg equilibrium ($p>0.05$). Four genotypes (Table IV), namely the *HLA-DRB1**15/*13, *HLA-DRB1**15/*04, *HLA-DRB1**09/*14, and *HLA-DRB1**04/*04 show tendency to association with a higher risk of developing MS, but after adjusted p value by FDR method these alleles and genotypes lost significance. No genotype appeared to confer protection for MS. (Table IV).

DISCUSSION

In European populations, the risk of developing MS is linked mainly to the HLA-DRB*1501 allele with some additional effects of other HLA alleles such as *HLA-DRB1**0301 and *HLA-DRB1**1303. This was demonstrated in a very broad study of 17456 cases and 30385 controls of 11 cohorts with European ancestry. The evidence in this study suggests that *HLA-DRB1**1501 exerts a dominant effect and *HLA-DRB1**0301 has a recessive effect on risk of RRMS. It was found that classical risk alleles for MS do not interact outside the HLA region and that, within HLA, the interaction by epistasis is low. The effects of protective alleles for MS in the evolution of the disease in European populations are also low, however the effects that have been demonstrated could be related to processes of cross reactivity that occur in the T cell. In conclusion for this study, in the European population the interactions between the

TABLE III
ASSOCIATION OF *HLA-DRB*1 ALLELES WITH RRMS SUBTYPE

HLA-DRB1 Allele	Count RRMS Patients (n=59)	Count Healthy Controls (n=434)	Unadjusted p value	Adjusted p value FDR method	Adjusted p value Bonferroni	OR	CI
16	2	20	1	0.671	1.0000	0.726	0.165-3.190
15	15	48	0.002	**0.013**	**0.0260**	2.741	1.419-5.295
14	11	94	0.596	0.646	1.0000	0.829	0.414-1.659
13	16	91	0.282	0.407	1.0000	1.403	0.755-2.604
12	1	1	0.225	0.18	1.0000	7.466	0.461-120.98
11	4	64	0.096	0.18	0.7680	0.420	0.147-1.200
10	0	13	0.382	0.289	1.0000	0.877	0.848-0.907
09	4	5	0.015	0.062	0.1800	6.240	1.627-23.94
08	5	93	0.019	0.062	0.1900	0.340	0.132-0.873
07	9	53	0.508	0.6	1.0000	1.294	0.602-2.783
04	41	238	0.033	0.086	0.2970	1.876	1.044-3.369
03	6	62	0.390	0.507	1.0000	0.679	0.280-1.647
01	4	86	0.015	0.062	0.1800	0.294	0.104-0.834

RRMS: Relapsing-remitting multiple sclerosis
Bold text = Significant ($p<0.05$)
p values of Chi-Square or Fisher exact test (when expected values <5).
CI = Confidence interval (95%)
FDR = False discovery rate

classical risk alleles within HLA as well as the risk alleles outside the HLA is minimal, suggesting a low interaction of polygenic epistasis in the modulation of risk alleles (25).

In Mexico, the frequency of *HLA-DRB1* alleles in our population in control subjects is similar to that reported in the general population of Guadalajara, Mexico (26) (Table I). Among MS patients frequency of the *HLA-DRB1**15 allele was twice more frequent than in the control group. This high frequency is similar to the 12-20% proportion observed in unselected Caucasian populations (27). Therefore, patients with RRMS included in our study appear to have a higher fraction of Caucasian ancestry. This observation agrees with the findings of a GWAS in MS patients and the general population of the country's central region, and implies that a greater European ancestry increases the risk of MS, whereas a greater genetic Amerindian component protects against this disease (28,29). In our population, the allele associated with increased MS risk were *HLA-DRB1**15 (p: 0.026;OR: 2.74; CI: 1.419-5.295). This allele has been re-

TABLE IV
PROBABLE RISK GENOTYPES FOR RRMS SUBTYPE

Genotypes	Frequency		Unadjusted p-value	Adjusted p value FDR method	Adjusted p value Bonferroni method	OR	CI
	RRMS patients (59)	Healthy Controls (434)					
15/13	4	2	0.002	0.068	0.155	15.71	2.81-87.76
15/04	7	9	0.001	0.068	0.081	6.35	2.27-17.78
09/14	2	1	0.040	0.680	1.000	15.19	1.356-170.2
04/04	7	19	0.033	0.680	1.000	2.94	1.18-7.328

RRMS: relapsing-remitting multiple sclerosis.
Frequency: the number of times the genotype appeared in the sample.
CI = Confidence interval (95%).
p = Fisher exact test.
FDR= False Discovery Rate.

cognized worldwide to confer risk of developing MS and other autoimmune diseases.

Gorodeszky *et al* reported in 1986 (30), the first study related to HLA and MS in Mexico and found association with the HLA-A3-B7-DR15 haplotype in MS patients with strong Caucasian ancestry in central México, but found no association for the *HLA-DRB1**15 allele alone. In 2000, Alvarado de la Barrera *et al.* (20), in a central Mexico population reported a small sample of 17 cases of familial and sporadic MS, they found high frequency of *HLA-DRB1**15 allele. However, in this study the association with the *HLA-DRB1**15 allele was not clear because of the small sample size and the mixture of sporadic cases with familial cases, in addition, only seven patients had mestizo ancestors (fathers and grandfathers). Subsequently, in a sample of 51 mestizos, Aláez *et al.* (11), reported the *HLA-DRB1**0403 and *HLA-DRB1**0802 alleles, but found no statistically significant association with the *HLA-DRB1**1501 allele. In similar manner to Aláez *et al.*(11), we found a tendency to increase the risk of RRMS of the *HLA-DRB1**04 allele (unadjusted p: 0.033, OR: 1.87, IC 1.044-3.369), however, we found the opposite for the *HLA-DRB1**08 allele (unadjusted p: 0.019, OR: 0.34, IC 1.044-3.369) which had a tendency to EMRR protection in our study.

In 2015, Flores *et al.* (21), for the first time, found a robust risk association for MS with alleles *HLA-DRB1**15 like we did in our population. Although it was not found for other risk alleles previously identified in the population of central Mexico. Flores *et al.*, also found a protection association for MS for allele *HLA-DRB1**13, they used very conservative p value adjustment by Bonferroni method for multiple comparisons, we also adjusted the value of P for multiple comparisons by the FDR method that has greater power than Bonferroni method, and yet we did not find protection association for allele *HLA-DRB1**13; This suggests that in Mexico, regional genetic heterogeneity is important in the determination of risk or protective alleles for MS. The heterogeneity in the effects of HLA alleles is reflected in the study of different diseases in Mexico, for example, a pattern

similar to that found in MS was found in risk alleles when HLA alleles were studied in leukemia in Mexican Mestizo children (31). However, in an inflammatory disease, clinically related to MS, called optic neuromyelitis (NMO), no common risk alleles were found between MS and NMO (32).

In our study we did not find the same risk or protection association profile for MS than in central Mexico, paradoxically, we found a tendency of protective effect for allele *HLA-DRB1**08 (OR: 0.34, unadjusted p value: 0.019, adjusted p value:0.062). However we find some similarities to central Mexico, allele *HLA-DRB1**04 had tendency to increase the risk for RRMS, alone, in homozygosis or when associated with *HLA-DRB1**15. Otherwise, *HLA-DRB1**01 appeared with tendency to protect against MS in our population (OR: 0.29; uncorrected p value: 0.015; adjusted p value: 0.062). These differences in risk allele profile could be due to genetic heterogeneity in Mexican Mestizo, diverse regional subtypes of *HLA-DRB1* alleles, some epistasis, epigenetic factors; and more importantly, the complex interaction between the alleles and the environment.

We determined that in western Mexico the allele *HLA-DRB1**15 increases the risk for development of MS; and probably *HLA-DRB1**09 and *HLA-DRB1**04 contribute to this risk too. Alleles *HLA-DRB1**08 and *HLA-DRB1**01 emerge with a tendency to being protective alleles; and the genotypes *HLA-DRB1**15/*13, *HLA-DRB1**15/*04, *HLA-DRB1**09/*14, and *HLA-DRB1**04/*04 have a tendency to increase the risk for development of RRMS. Further research is required, since we studied a small RRMS patient cohort.

The *HLA-DRB1* risk allelic variation in different regions of Mexico for MS, further illustrates the genetic heterogeneity of the Mexican population and the Caucasian influence in shaping the epidemiology of MS in Mexico. Accordingly, the higher incidence of MS in western Mexico, compared with other regions of the country, reflects a higher European ancestry therein.

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REFERENCES

1. **Porras-Betancourt M, Núñez-Orozco L, Plascencia-Álvarez N, Quiñones-Aguilar S, Sauri-Suárez S.** Esclerosis múltiple. Rev Mex Neuroci 2007;8(1):57-66.
2. **Ludwin SK.** The pathogenesis of multiple sclerosis: relating human pathology to experimental studies. J Neuropathol Exp Neurol 2006 ; 65(4):305-318.
3. **Fugger L, Friese MA, Bell JI.** From genes to function: the next challenge to understanding multiple sclerosis. Nat Rev Immunol 2009 ;9(6):408-417.
4. **Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG.** Multiple sclerosis. N Engl J Med 2000;343(13):938-952.
5. **Luetic G.** MS in Latin America. Int MS J 2008 ;15(1):6-11.
6. **De la Maza M, García J, Bernal J, Fuentes M.** A review of the epidemiology of multiple sclerosis in Mexico. Rev Neurol 2000 ;31(5):494-495.
7. **Velázquez-Quintana M, Macías-Islas MA, Rivera-Olmos V, Lozano-Zárate J.**

Multiple sclerosis in Mexico: a multicentre study. Rev Neurol 2003 ;36(11):1019-1022.

8. **Ramagopalan SV, Ebers GC.** Multiple sclerosis: major histocompatibility complexity and antigen presentation. Genome Med 2009 ;1(11):105.
9. **Lincoln MR, Montpetit A, Cader MZ, Saarela J, Dyment DA, Tiislar M, Ferretti V, Tienari PJ, Sadovnick AD, Peltonen L, Ebers GC, Hudson TJ.** A predominant role for the HLA class II region in the association of the MHC region with multiple sclerosis. Nat Genet 2005;37(10):1108-1112.
10. **Barcellos LF, Sawcer S, Ramsay PP, Baranzini SE, Thomson G, Briggs F, Cree BC, Begovich AB, Villoslada P, Montalban X, Uccelli A, Savettieri G, Lincoln RR, DeLoa C, Haines JL, Pericak-Vance MA, Compston A, Hauser SL, Oksenberg JR.** Heterogeneity at the *HLA-DRB1* locus and risk for multiple sclerosis. Hum Mol Genet 2006 ;15(18):2813-2824.
11. **Aláez C, Corona T, Ruano L, Flores H, Loyola M, Gorodezky C.** Mediterranean and Amerindian MHC class II alleles are associated with multiple sclerosis in Mexicans. Acta Neurol Scand 2005 ;112(5):317-322.
12. **Ramagopalan SV, Morris AP, Dyment DA, Herrera BM, DeLuca GC, Lincoln MR, Orton SM, Chao MJ, Sadovnick AD, Ebers GC.** The inheritance of resistance alleles in multiple sclerosis. PLoS Genet 2007 ;3(9):1607-1613.
13. **Stankovich J, Butzkueven H, Marriott M, Chapman C, Tubridy N, Tait BD, Varney MD, Taylor BV, Foote SJ; ANZgene Consortium., Kilpatrick TJ, Rubio JP.** *HLA-DRB1* associations with disease susceptibility and clinical course in Australians with multiple sclerosis. Tissue Antigens 2009 ;74(1):17-21.
14. **Weatherby SJ, Thomson W, Pepper L, Donn R, Worthington J, Mann CL, Davies MB, Fryer AA, Boggild MD, Young CA, Jones PW, Strange RC, Ollier WE, Hawkins CP.** *HLA-DRB1* and disease outcome in multiple sclerosis. J Neurol 2001;248(4):304-310.
15. **Fusco C, Andreone V, Coppola G, Luongo V, Guerini F, Pace E, Florio C, Pirozzi G, Lanzillo R, Ferrante P, Vivo P, Mini M, Macrì M, Orefice G, Lombardi ML.** *HLA-DRB1**1501 and response to copolymer-1 therapy in relapsing-remitting multiple sclerosis. Neurology 2001;57(11):1976-1979.
16. **Hoffmann S, Cepok S, Grummel V, Lehmann-Horn K, Hackermüller J, Stadler PF, Hartung HP, Berthele A, Deisenhammer F, Wassmuth R, Hemmer B.** *HLA-DRB1**0401 and *HLA-DRB1**0408 are strongly associated with the development of antibodies against interferon-beta therapy in multiple sclerosis. Am J Hum Genet 2008 ;83(2):219-227.
17. **Dyment DA, Herrera BM, Cader MZ, Willer CJ, Lincoln MR, Sadovnick AD, Risch N, Ebers GC.** Complex interactions among MHC haplotypes in multiple sclerosis: susceptibility and resistance. Hum Mol Genet 2005 ;14(14):2019-2026.
18. **Rubi-Castellanos R, Martínez-Cortés G, Muñoz-Valle JF, González-Martín A, Cerda-Flores RM, Anaya-Palafox M, Rangel-Villalobos H.** Pre-Hispanic Mesoamerican demography approximates the present-day ancestry of Mestizos throughout the territory of Mexico. Am J Phys Anthropol 2009 ;139(3):284-294.
19. **Martínez-Cortés G, Salazar-Flores J, Haro-Guerrero J, Rubi-Castellanos R, Velarde-Félix JS, Muñoz-Valle JF, López-Casamichana M, Carrillo-Ta-**

pia E, Canseco-Avila LM, Bravi CM, López-Armenta M, Rangel-Villalobos H. Maternal admixture and population structure in Mexican-Mestizos based on mtDNA haplogroups. Am J Phys Anthropol 2013 ;151(4):526-537**.**

20. **Alvarado-de la Barrera C, Zúñiga-Ramos J, Ruíz-Morales JA, Estañol B, Granados J, Llorente L.** HLA class II genotypes in Mexican Mestizos with familial and nonfamilial multiple sclerosis. Neurology 2000 ;55(12):1897-1900.
21. **Flores J, Granados J, Alonso E, Rito Y, Ortega-Hernández E, Mena-Hernández L, Corona T.** Presence of the *HLADR1**3 allele among Mexican Mestizos suggests a protective factor against relapsing-remitting multiple sclerosis (RRMS). Clin Neurol Neurosurg 2015;138:184-187.
22. **Vargas-Alarcón G, Granados J, Rodríguez-Pérez JM, Parga C, Pérez-Hernández N, Rey D, Zuñiga J, Arnaiz-Villena A.** Distribution of HLA class II alleles and haplotypes in Mexican Mestizo population: comparison with other populations. Immunol Invest 2010 ;39(3):268-283.
23. **Duquette P, Décary F, Pleines J, Boivin D, Lamoureux G, Cosgrove JB, Lapierre Y.** Clinical sub-groups of multiple sclerosis in relation to HLA: DR alleles as possible markers of disease progression. Can J Neurol Sci 1985 ;12(2):106-110.
24. **Glickman ME, Rao SR, Schultz MR.** False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies. J Clin Epidemiol 2014 ;67(8):850-857.
25. **Moutsianas L, Jostins L, Beecham AH, Dilthey AT, Xifara DK, Ban M, Shah TS, Patsopoulos NA, Alfredsson L, Anderson CA, Attfield KE, Baranzini SE, Barrett J, Binder TMC, Booth D, Buck D, Celius EG, Cotsapas C, D'Alfonso S, Dendrou CA, Donnelly P, Dubois B, Fontaine B, Fugger L, Goris A, Gourraud PA, Graetz C, Hemmer B, Hillert J; International IBD Genetics Consortium (IIBDGC)., Kockum I, Leslie S, Lill CM, Martinelli-Boneschi F, Oksenberg JR, Olsson T, Oturai A, Saarela J, Søndergaard HB, Spurkland A, Taylor B, Winkelmann J, Zipp F, Haines JL, Pericak-Vance MA, Spencer CCA, Stewart G, Hafler DA, Ivinson AJ, Harbo HF, Hauser SL, De Jager PL, Compston A, McCauley JL, Sawcer S, McVean G.** Class II HLA interactions modulate genetic risk for multiple sclerosis. Nat Genet 2015 ;47(10):1107-1113.
26. **Cortes LM, Baltazar LM, Perea FJ, Gallegos-Arreola MP, Flores SE, Sandoval L, Olivares N, Lorenz MG, Xu H, Barton SA, Chakraborty R, Rivas F.** HLA-DQB1, -DQA1, -DRB1 linkage disequilibrium and haplotype diversity in a Mestizo population from Guadalajara, Mexico. Tissue Antigens 2004 ;63(5):458-465.
27. **Dean G, Yeo TW, Goris A, Taylor CJ, Goodman RS, Elian M, Galea-Debono A, Aquilina A, Felice A, Vella M, Sawcer S, Compston DA.** *HLA-DRB1* and multiple sclerosis in Malta. Neurology 2008;70(2):101-105.
28. **Ordoñez G, Romero S, Orozco L, Pineda B, Jiménez-Morales S, Nieto A, García-Ortiz H, Sotelo J.** Genomewide admixture study in Mexican Mestizos with multiple sclerosis. Clin Neurol Neurosurg 2015;130:55-60.
29. **Flores J, González S, Morales X, Yescas P, Ochoa A, Corona T.** Absence of multiple sclerosis and demyelinating diseases among Lacandonians, a pure Amerindian ethnic group in Mexico. Mult Scler Int 2012;2012:292631.
30. **Gorodezky C, Najera R, Rangel BE,**

Castro LE, Flores J, Velázquez G, Granados J, Sotelo J. Immunogenetic profile of multiple sclerosis in Mexicans. Hum Immunol 1986 ;16(4):364-374.

31. **Morrison BA, Ucisik-Akkaya E, Flores H, Alaez C, Gorodezky C, Dorak MT.** Multiple sclerosis risk markers in HLA-DRA, HLA-C, and IFNG genes are associated with sex-specific childhood leukemia risk. Autoimmunity 2010;43(8):690-697.
32. **Alonso VR, de Jesús Flores Rivera J, Garcí YR, Granados J, Sánchez T, Mena-Hernández L, Corona T.** Neuromyelitis optica (NMO IgG+) and genetic susceptibility, potential ethnic Influences. Cent Nerv Syst Agents Med Chem 2016; Feb 28. [Epub ahead of print].