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HLA class II polymorphism in Latin American patients with multiple sclerosis

Olga-Lucía Rojas [a,b,1], Adriana Rojas-Villarraga [a,1], Paola Cruz-Tapias [a], Jorge Luis Sánchez [c], Juan-Camilo Suárez-Escudero [c], Manuel-Alfonso Patarroyo [d,e], Juan-Manuel Anaya [a,*]

[a] Center for Autoimmune Diseases Research (CREA), School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia
[b] Unidad de Inmunología, School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia
[c] Instituto Neurológico de Antioquia, INDEA, Medellín, Colombia
[d] Fundación Instituto de Inmunología de Colombia (FIDIC), Bogotá, Colombia
[e] School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia

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ABSTRACT

Objective: To identify HLA-DRB1 alleles contributing to susceptibility to multiple sclerosis (MS) in a Colombian population and to estimate the common effect size of HLA class II on MS susceptibility in Latin American populations through a meta-analysis.
Methods: A total of 65 Colombian patients with MS and 184 matched controls were included. HLA-DRB1 typing was done using the sequence-specific oligonucleotide probe method. A bivariate and a multivariate logistic regression analyses were done. Case-control studies performed in Latin America were searched up to January 2009 through a systematic review of the literature. Effect summary odds ratios (ORs) and 95% confidence intervals (CIs) were obtained by means of the random effect model.
Results: A total of 464 cases and 2581 controls from 7 studies and the results of the present study in Colombians were analyzed. HLA-DRB1*15 (OR: 2.3; 95% CI: 1.68–3.07; $p<0.001$) and HLA-DQB1*06 (OR: 2.2; 95% CI: 1.54–3.07; $p<0.001$) groups as well as DRB1*1501 (OR: 2.6; 95% CI: 1.67–4.02; $p<0.001$), DRB1*1503 (OR: 2.2; 95% CI: 1.39–3.62; $p=0.001$) and DQB1*0602 (OR: 2.5; 95% CI: 1.66–3.71; $p<0.001$) alleles were found to be risk factors for MS. The myelin basic protein immunodominant sequence $_{221}$VHFFKNIVT$_{229}$ was predicted to strongly and simultaneously bind to HLA-DRB1*1501 and *1503.
Conclusion: The current study highlights the effect size of HLA class II in MS in Latin America and confirms similar allelic risk factors across diverse populations. Receptor-ligand interactions in the HLA-antigenic peptide complex could have potential predictive and therapeutical implications.

Contents

* Corresponding author. Center for Autoimmune Diseases Research (CREA), School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia, Carrera 24 # 63C-69 Bogotá, Colombia. Tel.: +57 1 349 9650; fax: +57 1 349 9410.
E-mail address: anayajm@gmail.com (J.-M. Anaya).
[1] Both authors contributed equally.

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1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system and is one of the most disabling autoimmune diseases (ADs) [1]. The precise etiology of MS is not well understood yet; however, both genetic and environmental factors are implicated. Several Human Leukocyte Antigens (HLA) have been associated with MS, but this association may vary according to the ethnic and geographical characteristics of the patients [2–4]. A primary role for HLA class I, independent of class II, has been suggested in the etiology of MS [3]. Within the class II region, the HLA-DRB1*1501 and HLA-DQB1*0602 alleles, which are in linkage disequilibrium, are the main risk alleles associated with MS in Caucasians [4]. HLA-DRB1*15 has been associated with younger age at onset and worse Expanded Disability Status Scale score [5] as well as with severe morbidity in patients with primary progressive MS [6].

Comparisons between geographical areas and ethnic groups are essential to determine the influence of environmental and genetic factors on the development of ADs. Despite the low prevalence of MS in Latin America, genetic studies in populations belonging to this geographical area offers a unique opportunity for examining the predisposition to develop MS because of the negative influence contributed by the admixture of Amerindians, Europeans and Negroids occurring 10–20 generations ago. This may resolve racial effects from genetic association with major genes [7]. The study of HLA as one of the major locus contributing to ADs will allow us to understand its common or specific influence on these diseases [2,8]. Thus, the purpose of this study was to analyze the role of HLA-DRB1 gene in susceptibility to MS in a Colombian population and to estimate the common effect size of HLA class II on the disease across Latin America populations through a meta-analysis.

2. Materials and methods

2.1. Study population

A total of 65 adult patients (10 men and 55 women) with clinically defined MS diagnosed by using McDonald's criteria [9] and evaluated in a specialized neurology center were included in this study. A total of 184 matched, unrelated, healthy individuals from the same community were included as controls. All patients and controls were born in Medellin, Antioquia, Colombia or its surroundings, a genetically well-defined population known as the "Paisa community" [7,10]. All patients gave informed consent to their inclusion in this study, which was approved by the local Ethics Committee.

2.2. DNA extraction and HLA typing

Genomic DNA was extracted from 10 mL of EDTA-anticoagulated peripheral blood or from 4 mL of saliva from each individual. To isolate the DNA from the blood, the standard salting out method [11] or the PROBE protocol was used. DNA purification from saliva was done by using Oragene DNA Self-Collection Kit (DNA Genotek Inc, Ottawa, Canada) according to the manufacturer's specifications.

Class II HLA-DRB1 typing was done by using the DRB1 Kit (INNO-LiPA Kits from Innogenetics NV, Belgium) as previously described [10].

2.3. Search strategy and selection criteria

A systematic review of Electronic Databases (MEDLINE, PubMed, SciELO, BIREME, EMBASE, Cochrane and LILACS) was done independently by two experts. The final date for inclusion was January 2009. The search only included publications on HLA-Class II alleles and susceptibility to MS in Latin America published in any of these three languages: Spanish, English or Portuguese. The search strategy used MeSH terms and the text words: "Multiple Sclerosis" [Major] and HLA DR/DQ antigens in combination with all Latin American countries, including Caribbean islands [MeSH]. For the search in the Spanish and Portuguese databases, the DeCS terms (Descriptores en Ciencia de la Salud): "Esclerosis Múltiple", "Antígenos HLA" and "Complejo Mayor Histocompatibilidad" were used. No other criteria were taken into account.

The inclusion criteria were the following: 1) MS diagnosis established using Poser's or Macdonald's criteria [9,12]; 2) case-control design of the study; 3) use of molecular techniques to determine HLA polymorphisms; 4) publication of sufficient information to calculate odds ratios (ORs); 5) being focused on a well defined Latin American population, and; 6) manuscript's publication in a peer-reviewed journal as full paper. Summaries or abstracts were not accepted.

2.4. Data extraction

The following information was collected from each study: author, year of publication, a detailed description of ethnicity in the studied population, HLA typing technique used, MS type, diagnosis criteria for MS, Hardy-Weinberg (HW) test information (if available), and total number of cases, controls, individuals and/or alleles per genotype reported in tables as well as in the manuscript's text. Serological specificities for each allele reported at the 13th International Histocompatibility Workshop and Conference were used to group data from all studies (expert assigned nomenclature) [13].

2.5. Statistical analysis

2.5.1. Colombian "Paisa population"

First, the association between genetic data results at the allelic level in the "Paisa population" and MS were assessed by bivariate analyses. χ^2 tests or Fisher's exact tests were applied to dichotomous factors. Next, for each specific allele that was significantly associated with MS in bivariate analyses, a gender-adjusted multivariate logistic regression model was estimated. This model considered MS as the dependent variable and all the alleles that were significantly associated with MS in the bivariate analyses as independent variables. Nagelkerke *R*-square was calculated to determine the proportion of variability in the logistic regression model. Adjusted OR (AORs) that measured the effect size of specific alleles on MS were computed together with their 95% confidence intervals (CI). The adequacy of logistic models was assessed using the Hosmer–Lemeshow goodness-of-fit test with the null hypothesis that the data were generated by the fitted model. All of the statistical analyses were done by using the Statistical Package for the Social Sciences (SPSS, v.15, Chicago, IL).

2.5.2. Meta-analysis

Data were analyzed using the Comprehensive Meta-Analysis version 2 program (Biostat, Englewood, NJ, 2004). Calculations were carried out for each HLA-DRB1 and HLA-DQ allele using low or high resolution based on information available in each article. ORs were grouped by weighing individual OR by the inverse of their variance. For each allele, the final effect OR and 95% CI were obtained by means of both random and fixed effect models. The selection of the computational model was done based on the expectation that the studies shared a common effect size. The random effect model was preferred because it assumes that there is a distribution of true effect sizes

rather than one true effect and assigns a more balanced weight to each study. It was also used because all the studies were considered to be functionally unequal. Heterogeneity was calculated by means of Cochran's (Q) and Higgins's (I^2) tests. The I^2 test showed the proportion of observed dispersion that was real rather than spurious, and was expressed as a ratio ranging from 0% to 100%. I^2 values of 25%, 50% and 75% were qualitatively classified as low, moderate and high, respectively. A significant Q-statistic ($p<0.10$) indicated heterogeneity across studies. Publication bias was determined using Funnel plots and Egger's regression asymmetry tests.

The expected statistical power of each study to detect a true association between HLA-DRB1 alleles and MS, or between HLA-DQA1 and HLA-DQB1 alleles with MS was calculated using PS Power and Sample Size Calculations Version 2.1.31 (Copyright© 2004 by William D. Dupont and Walton D. Plummer, Vanderbilt Biostatistics, Nashville, TN). We used a 0.05 level of significance, expecting a probability of exposure in cases relative to controls (i.e. OR) of 1.72 and 3.58 for HLA-DR15; 1.94 and 6.36 for HLA-DRB1*1501; 1.53 and 8.88 for HLA-DRB1*1503; 1.5 and 2.87 for HLA-DQB1*06; and 1.76 and 3.73 for HLA-DQB1*0602. These ORs represented the 25 and 75 percentiles of the distribution of effect sizes for each allele or group.

3. Results

3.1. Study population

To determine the association of HLA-DRB1 and MS, a total of 130 case alleles and 368 controls alleles were analyzed, with a ratio of 2.8 controls per case. A logistic regression analysis was applied since female gender was significantly associated with the disease (χ^2 11.17 $p=0.001$). The adjusted analysis showed that the DRB1*0103 allele and DRB1*15 group were risk factors whereas the DRB1*0701 allele and DRB1*04 group were protective factors (Table 1). The logistic model fitted well (Hosmer–Lemeshow p value >0.05).

3.2. Studies included

The initial search strategy allowed us to identify 18 studies for potential inclusion which included review articles. Among this group, 12 association studies related to HLA-DRB1, HLA-DQA1 and/or HLA-DQB1 polymorphisms and susceptibility to MS were identified. A total of 8 articles on the HLA-Class II region fitted our selection criteria (Table 2) [7,14–20]. One article [21] was excluded because of duplicate information; another article [22] was excluded because it lacked sufficient information to do OR calculations. Other study was excluded because it included a non-Latin American population [23] and another one because it did not use molecular techniques for HLA typing and the MS diagnosis criteria was UCLA-VA [24]. The results of the present study on Paisa population were included for meta-analysis of HLA-DRB1 alleles. HW equilibrium data was only reported in 4 series, including ours.

3.3. HLA meta-analytic association

An association between HLA-DRB1*15 and HLA-DQB1*06 groups and MS was demonstrated (Fig. 1). At the allelic level, DRB1*1501, DRB1*1503 and DQB1*0602 were found to be risk factors for MS (Fig. 1). No protective factors were found. The meta-analysis of HLA-DQA1 alleles was done based on data reported in 5 articles, one of which included a previous study done on the Paisa community [7,15–17,20] but no statistically significant associations were established (data not shown).

Heterogeneity was not significantly observed for the HLA-DQB1*06 group and for the DRB1*1501 and DRB1*1503 alleles (Table 2). Moderate heterogeneity for the HLA-DRB1*15 group as well as for

Table 1
HLA-DRB1 allele frequencies in Colombian patients with MS and controls.

DRB1 Group[a]	DRB1 alleles	Allele frequency		Test of association			R^2
		Cases	Controls	AOR	95% CI	p-value	
DRB1*01		0.146	0.084			NS	
	DRB1*0101	0.008	0.024			NS	
	DRB1*0102	0.054	0.033			NS	
	DRB1*0103	0.062	0.005	9.961	2.08–47.707	0.004	0.06
	DRB1*0104	0	0.005			NS	
DRB1*03[b]		0.131	0.087			NS	
	DRB1*0301	0.062	0.027	0.512	0.29–0.906	0.022	0.052
	DRB1*0304	0	0.003			NS	
	DRB1*0305	0	0.003			NS	
DRB1*04		0.131	0.226			NS	
	DRB1*0401	0	0.003			NS	
	DRB1*0402	0.008	0.008			NS	
	DRB1*0403	0	0.011			NS	
	DRB1*0404	0	0.030			NS	
	DRB1*0405	0	0.003			NS	
	DRB1*0407	0.038	0.041			NS	
	DRB1*0408	0	0.011			NS	
	DRB1*0410	0.008	0.016			NS	
	DRB1*0411	0.023	0.008			NS	
	DRB1*0422	0	0.003			NS	
	DRB1*0435	0.008	0			NS	
	DRB1*0440	0.008	0			NS	
DRB1*07		0.092	0.106			NS	
	DRB1*0701	0.023	0.084	0.264	0.079–0.883	0.031	0.054
	DRB1*0706	0	0.005			NS	
	DRB1*0708	0.008	0			NS	
DRB1*08		0.069	0.065			NS	
	DRB1*0801	0.015	0.019			NS	
	DRB1*0802	0.015	0.014			NS	
	DRB1*0804	0	0.008			NS	
	DRB1*0817	0.008	0			NS	
DRB1*09		0.008	0.022			NS	
	DRB1*0901	0.008	0.019			NS	
DRB1*10		0.023	0.014			NS	
	DRB1*1001	0.023	0.014			NS	
DRB1*11		0.046	0.041			NS	
	DRB1*1101	0.031	0.016			NS	
	DRB1*1102	0.008	0.003			NS	
	DRB1*1104	0	0.003			NS	
	DRB1*1121	0	0.003			NS	
DRB1*12		0	0.005			NS	
	DRB1*1202	0	0.003			NS	
DRB1*13[b]		0.100	0.158			NS	
	DRB1*1301	0	0.016			NS	
	DRB1*1302	0.023	0.024			NS	
	DRB1*1303	0.008	0.003			NS	
	DRB1*1317	0.008	0			NS	
	DRB1*1334	0.008	0			NS	
DRB1*14[b]		0.046	0.043			NS	
	DRB1*1401	0	0.008			NS	
	DRB1*1402	0	0.005			NS	
	DRB1*1407	0.008	0			NS	
	DRB1*1421	0	0.003			NS	
	DRB1*1433	0.008	0			NS	
	DRB1*1439	0	0.003			NS	
DRB1*15[b]		0.162	0.098	2.45	1.44–4.17	0.039	0.047
	DRB1*1501	0.062	0.033			NS	
	DRB1*1502	0	0.005			NS	
	DRB1*1503	0.023	0.008			NS	
	DRB1*1505	0.008	0			NS	
	DRB1*1510	0	0.005			NS	
DRB1*16		0.031	0.041			NS	
	DRB1*1601	0	0.005			NS	
	DRB1*1602	0.031	0.027			NS	

Data correspond to 65 patients and 184 controls analyzed for HLA-DRB1 gene taking into account the number of chromosomes (i.e. 2 N).
AOR: Adjusted odds ratio (by gender); CI: confidence interval; R^2: Nagelkerke R-square, which determines the proportion of variability accounted by the logistic regression model; NS: Not significant.
[a] Expert assigned serologic group classification [13].
[b] DRB1*0305, DRB1*1303, DRB1*1433 and DRB1*1510 alleles were included in the former WHO HLA nomenclature but are not considered in the specific expert assigned group [13].

Table 2
Individual characteristics of the studies included in the meta-analysis.

HLA-DRBI1 and HLA-DQB1 polymorphism associated with MS					DR alleles							
					DR15				DRBI*1501			
Study	Population	Sample Size	Cases (Allelic frequency)	Control per case	Relative weight		Expected power[a] E(DR15) [b] = 0.088		Relative weight		Expected power[a] E(DRBI*1501) [b] = 0.051	
					Fixed	Random	$\psi = 1.72$	$\psi = 3.58$	Fixed	Ramdom	$\psi = 1.89$	$\psi = 5.18$
Alves-Leon S. et al	African Brazilian	264	88	2	14.043	14.136	0.285	0.938	4.42	6.290	0.269	0.969
Alves-Leon S. et al	White Brazilian	264	80	2.3	12.894	13.528	0.28	0.929	31.970	24.650	0.266	0.963
Guimaraes Brum, et al	Mulato Brazilian	114	58	0.96	9.095	11.089	0.146	0.665	7.640	9.880	0.134	0.745
Guimaraes Brum, et al	White Brazilian	340	168	1.02	21.669	17.192	0.348	0.986	23.560	21.110	0.314	0.996
Aláez C, et al	Mexican Mestizos	448	102	3.39	4.589	6.928	0.376	0.981	7.410	9.650	0.358	0.996
Alvarado-de la Barrera C, et al	Mexican Mestizos	232	34	5.82	4.345	6.645	0.207	0.755	11.180	13.170	0.211	0.835
Patrucco L, et al	Caucasian Argentinean	2554	122	19.93	19.66	16.522	0.527	0.998	NA	NA	NA	NA
Current study	Colombian Paisa	498	130	2.83	13.700	13.960	0.428	0.993	13.830	15.260	0.401	0.998
Kelly MA. et al	Afro-Caribbean	228	54	3.22	NA	NA	NA	NA	NA	NA	NA	NA
Carvalho A, et al	Caucasic and African Brazilian	160	52	2.07	NA	NA	NA	NA	NA	NA	NA	NA
Heterogeneity					*Q*	*df*	*p*-value	I^2	*Q*	*df*	*p*-value	I^2
					12.9	7	0.07	45.78	8.82	6	0.184	30.01

NA: information not available from published data, MS: Multiple Sclerosis, *Q*: Cochran's test, I^2: Higgins' test, *df*: degrees of freedom, ψ: OR.
[a] $\alpha = 0.05$.
[b] Corresponds to the ratio between controls count per allele and total controls.

the DQB1*0602 allele was observed (Table 2). As mentioned before, a random effect model was used to calculate the common effect sizes.

3.4. Study quality

No type of 'grey literature' was included as it is not peer reviewed and did not meet the inclusion criteria. There was no evidence of publication bias in the current meta-analysis according to the Funnel plot and Egger's regression test. For DRB1*15, the *t* value was 0.056 [degrees of freedom (*df*) = 6; $p = 0.95$]. For DQB1*06, *t* value was 1.69 ($df = 4$; $p = 0.16$). For DRB1*1501, the *t* value was 0.44 ($df = 5$; $p = 0.67$). For DRB1*1503, the *t* value was 0.97 ($df = 4$; $p = 0.38$) and for DQB1*0602, the *t* value was 1.52 ($df = 4$; $p = 0.20$).

4. Discussion

Results in the Colombian population (i.e. Paisa community) indicated that the HLA-DRB1*15 and DRB1*0103 were risk factors for MS whereas DRB1*0701 was protective. It has been suggested that HLA-DRB1*0103 is a necessary but insufficient risk allele for MS [25]. DRB1*0701 was observed to be a protective factor against MS in the context of DRB1*0701-DQA1*0201-DQB1*0201 haplotype in Iceland [26]. Nevertheless, since the sample size was not as large as expected, our data were included in the meta-analysis. By using this strategy, an association between HLA-DRB1*1501, DRB1*1503 and DQB1*0602 alleles and MS was found in Latin Americans (Fig. 1), as has been shown in European-descent populations [2–4]. This is an important finding rather than a confirmatory one since the extensive admixture of Latin Americans anticipated a different pattern of HLA association with MS.

HLA-DRB1*1501–DQB1*0602 haplotype is known to play a major role in the pathogenesis of MS [4]. $CD4^+$ T helper cells are associated with many aspects of the autoimmune response in MS. These cells are specifically activated by some autoantigens derived from some proteins such as myelin basic protein (MBP) and presented by MHC-class II system molecules [27]. Previous studies aiming at identifying immunodominant epitopes within MBP being recognized by T-cells from MS patients have mainly mapped two peptides: $MBP_{84\text{-}102}$ and $MBP_{143\text{-}168}$ [28,29]. $MBP_{84\text{-}102}$ was found to be both DR2- and DQw1-restricted. The former corresponds to some alleles belonging to the current HLA-DRB1*15 group, while the latter corresponds to some alleles from either HLA-DQB1*05 or *06 [13]. The identification of MBP peptides that bind to the most common alleles associated with MS and described here is of pivotal importance for understanding functional and biological aspects of MS and HLA class II associations. Therefore, by using the computational method NetMHCIIPan (available at http://www.cbs.dtu.dk/services/NetMHCIIpan/), we observed that the MBP immunodominant sequence $_{218}$NPVVHFFKNIVTPRT$_{232}$, numbered according to the MBP sequence found under GenBank accession P02686, was the only peptide predicted to strongly and simultaneously bind to HLA-DRB1*1501, *1503 and *0103 alleles. This may be explained by the fact that a number of HLA-DR types have overlapping peptide-binding registers [30]. It seemed odd to us that peptide positions 84-102 and 143-168, previously reported as immunodominant experimentally, were not predicted as binders to any of the above-mentioned alleles [28,29], however, when we retrieved the sequences reported originally, we found that peptide 84-102 has the sequence DENPVVHFFKNIVTPRTPP [28], which overlaps almost entirely with our predicted immunodominant epitope.

Since different HLA molecules have been grouped together into supertypes due to their similar specificities [31], some of the peptides that bind to one allele in a supertype can bind to all alleles within this supertype. Accordingly, based on HLA-DR clustering from NetMHCIIPan predictions [32], alleles related to DR15, DR7 and DR1 supertypes share some pocket specificities. By using sequence logos [33], some similarities and differences for HLA DRB1*1501 and DRB1*0701 alleles were found. The logos for HLA DRB1*1501 and *0701 show a similar preference for hydrophobic amino acids (Leucine) in pockets 7 and 9, while the residues are different in pockets 1 and 4 [33], which might explain why the former allele confers susceptibility to MS, while the latter is associated with protection. Likewise, DRB1*1501 and *1503 show similar amino acid preferences in their peptide binding pockets, whereas *0103 specificity is different and yet, this allele is predicted to strongly bind the same immunodominant epitope. This can be explained by the different binding registers used by the alleles; the binding core predicted for both HLA DRB1*1501 and *1503 is $_{221}$VHFFKNIVT$_{229}$ while for HLA DRB1*0103, the binding core

DR alleles				DQ alleles							
DRBI*1503				DQ6				DQB1*0602			
Relative weight		Expected power[a] E(DRBI*1503) $b=0.030$		Relative weight		Expected power[a] E(DQ6) [b] $=0.280$		Relative weight		Expected power[a] E(DQBI*0602) [b] $=0.16$	
Fixed	Random	$\psi=1.53$	$\psi=8.88$	Fixed	Random	$\psi=1.5$	$\psi=2.87$	Fixed	Random	$\psi=1.76$	$\psi=3.73$
59.853	59.853	0.114	0.993	26.829	23.062	0.314	0.976	24.173	20.858	0.431	0.993
2.665	2.665	0.115	0.991	24.733	22.052	0.303	0.97	21.077	19.598	0.419	0.99
13.058	13.058	0.07	0.862	NA	NA	NA	NA	NA	NA	NA	NA
11.661	11.661	0.114	1	NA	NA	NA	NA	NA	NA	NA	NA
3.947	3.947	0.145	0.999	11.227	13.213	0.406	0.995	8.002	11.068	0.551	0.999
NA	NA	NA	NA	7.610	9.750	0.194	0.804	7.678	10.749	0.279	0.894
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
8.816	8.816	0.155	1	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	13.136	14.750	0.243	0.912	21.137	19.624	0.342	0.962
NA	NA	NA	NA	16.416	17.173	0.21	0.861	17.934	18.102	0.29	0.93
Q	*df*	*p*-value	I^2	*Q*	*df*	*p*-value	I^2	*Q*	*df*	*p*-value	I^2
4.92	5	0.42	0	7.54	5	0.183	33.75	9.29	5	0.098	46.19

starts three residues after: $_{224}$FKNIVTPRT$_{232}$. Noteworthy, VHFFKNIVT sequence has been considered as a "peptide therapy" for MS [34].

A multipolar molecular electrostatic potential analysis of the receptor–ligand interactions involved in the formation of the complex between HLA class II molecules and antigenic peptides (HLA-DRB1*1501–MBP) disclosed that amino acids occupying the polymorphic positions beta13R, beta26F, beta28D, beta9W, beta74A, beta47F and beta57D are relevant for this receptor–ligand interaction [35], which could have potential predictive and therapeutical implications.

Epstein-Barr virus (EBV) infection and elevated humoral immune responses to EBV are associated with MS [36,37]. EBV nuclear antigen 1 (EBNA1)-specific $CD4^+$ Th1 cells are selectively expanded in MS patients, and these have the ability to cross-recognize MS-associated myelin antigens [38]. Carriers of the HLA-DR15 with elevated anti-EBNA-1 antibody titers may have a markedly increased risk of MS [39]. Thus, HLA-DRB1*1501 could predispose for selection of EBNA1 cross-reactive epitopes and increased total number of cross-reactive EBNA1-specific T cells, generated in susceptible HLA individuals, might contribute to the development of MS [4,38].

In conclusion, the current study highlights the effect size of HLA class II alleles associated with susceptibility to MS in Latin Americans and confirms similar allelic risk factors across diverse populations. Studies in which ancestry is systematically examined are necessary to evaluate whether or not Amerindian admixture confers protection against MS development in Latin Americans [40].

Take-home messages

- HLA-DRB1*1501, DRB1*1503 and DQB1*0602 alleles influence susceptibility to MS in Latin Americans.
- The MBP immunodominant sequence $_{221}$VHFFKNIVT$_{229}$ was predicted to strongly and simultaneously bind to HLA-DRB1*1501, *1503.
- Receptor-ligand interactions in the HLA-antigenic peptide complex (HLA-DRB1*1501-MBP) could have potential predictive and therapeutical implications.

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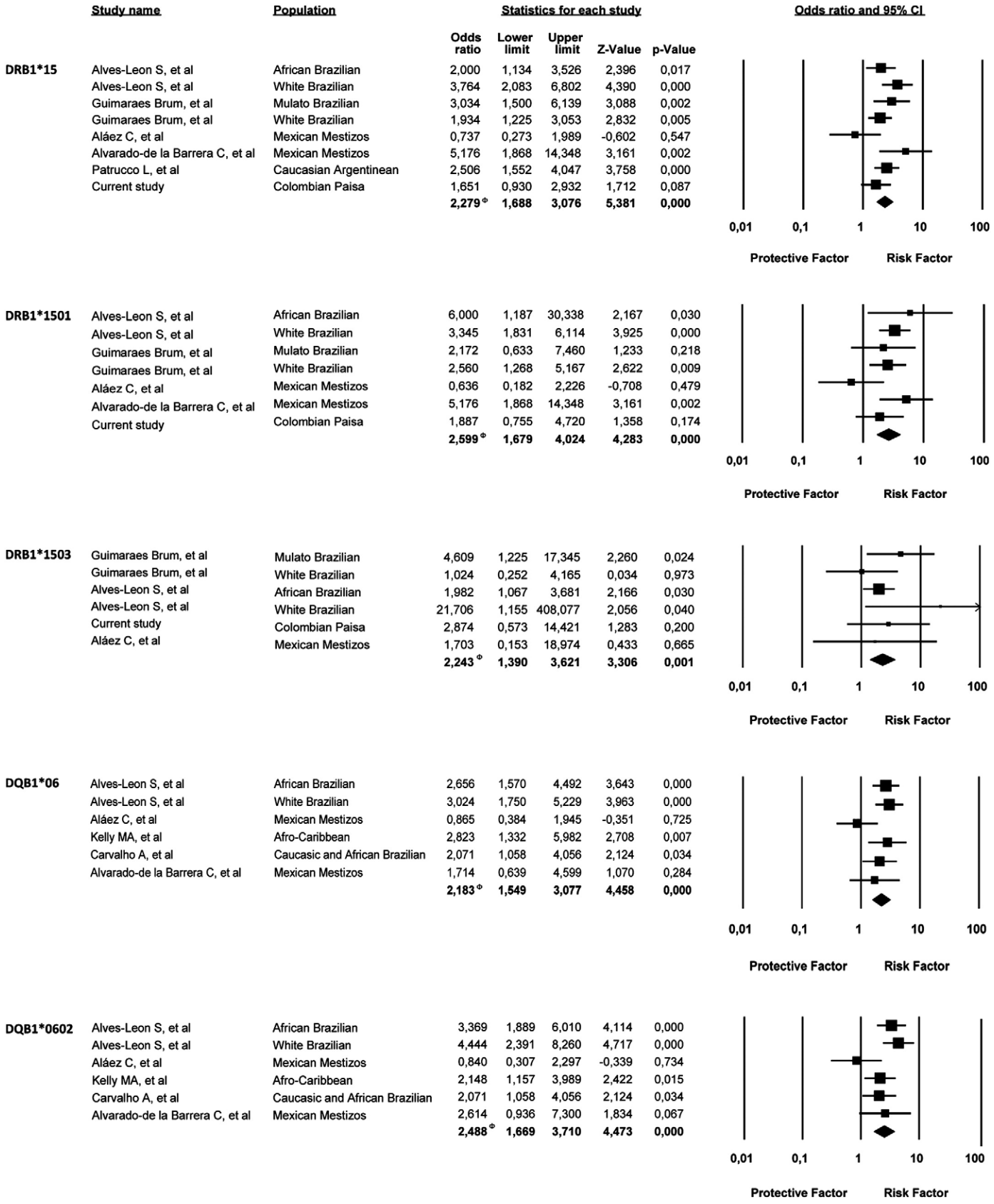

Fig. 1. Meta-analysis forest plot of MS in Latin America. Meta-analysis forest plot for significant alleles associated with MS: HLA-DRB1*15, HLA-DQB1*06, HLA-DRB1*1501, HLA-DRB1*1503 and HLA-DQB1*0602. Each plot shows the effect size and precision for individual studies and for the combined effect calculated by the Random Model. Filled squares are proportional in size to study weights. [Φ]Global OR for each allele.

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Circulating endothelial cells and angiogenic proteins in patients with systemic lupus erythematosus

The aim of this study was to assess absolute counts of different subpoulatins of circulating endothelial cells (CEC) in patients with active systemic lupus erythematosus (SLE). **Robak E. et al (Lupus 2009; 18: 332-41).** The authors investigated a potential correlation of CEC numbers with serum levels of angiogenic proteins as well as with clinical and laboratory symptoms of the disease. For the first time in SLE, CEC were enumerated directly, by the 'single platform' method. Resting (rCEC), activated (aCEC) and progenitor (pCEC) endothelial cells were identified in patients with SLE and healthy volunteers using four-colour flow cytometry. Serum concentrations of angiogenic proteins (vascular endothelial growth factor, placental growth factor (PIGF), soluble vascular cell adhesion molecule and endoglin) were evaluated by ELISA. The SLE activity was scored according to the Systemic Lupus Activity Measure system. The authors found that total CEC number in patients with SLE was significantly higher (median 14.2/microl) than in the control group (median 3.3/microl) ($p < 0.0001$). Absolute counts of aCEC, rCEC and pCEC (medians 4.9/microl, 6.8/microl and 2.3/microl, respectively) were also higher in patients with SLE than in healthy persons (medians 0.9/microl, 1.6/microl and 0.1/microl, respectively), with $p < 0.0001$ for all comparisons. There was no correlation between CEC or their subpopulations and SLE activity. Strong positive correlations were found between CEC, rCEC and pCEC number and serum levels of PIGF. Moreover, aCEC, rCEC and pCEC counts were significantly higher in SLE patients with leucopenia. In conclusion, our results show that absolute CEC counts and angiogenic proteins levels are elevated in patients with SLE as compared with healthy controls. These data may suggest that angiogenic process is involved in the pathogenesis of this disease.

HLA-E gene polymorphism associated with susceptibility to Kawasaki disease and formation of coronary artery aneurysms

Kawasaki disease (KD) is a pediatric systemic vasculitis of unknown cause for which a genetic influence is supposed. However matching susceptible genes to KD with specific clinical manifestations remains controversial. The purpose of the study performed by **Lin YJ, et al. (Arthritis Rheum 2009; 60:604-610)** was to identify possible genetic variants in the major histocompatibility complex (MHC) region that are associated with KD and the development of coronary artery aneurysms (CAAs) in a Taiwanese population. The 168 genetic variants covering the MHC locus were analyzed in an association study of a Taiwanese cohort of 93 KD patients and 680 unrelated healthy children matched for sex and age with the study patients. They found that eleven single-nucleotide polymorphisms (SNPs) were associated with the occurrence of KD. The SNP located at the 3'-untranslated region of HLA-E (rs2844724) was highly associated ($P < 1 \times 10(-7)$). In addition, the frequency of the C allele was higher in KD patients without CAAs than in controls ($P < 0.001$) due to a significantly increased frequency of the CC and CT genotypes. Plasma levels of soluble HLA-E were significantly higher in KD patients than in controls regardless of the presence of CAAs. Furthermore, there was a trend toward higher plasma levels of soluble HLA-E in KD patients with the CT and TT genotypes of the HLA-E gene polymorphism. Their results suggest that the HLA-E gene polymorphism may play a role in the pathogenesis of KD.