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Review

HLA-Class II in Latin American patients with type 1 diabetes

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ABSTRACT

Objective: To identify and estimate the common effect size of HLA-Class II contributing to susceptibility on T1D in Latin America (LA) through a meta-analysis.
Methods: A systematic review of the literature searching for all HLA-Class II alleles and susceptibility for T1D case–control studies performed in LA was made up to October 2009. Effect summary ORs and 95% CI were obtained by means of the random effect model. A prediction model that identifies peptides binding to HLA-DR alleles that were significantly associated with T1D throughout the meta-analysis was done.
Results: 21 studies were included (1138 cases and 1920 controls). DRB1*0301 (OR: 9.65; 95% CI: 5.69–16.36; $p<0.0001$), DRB1*1201 (OR: 4.84; 95% CI: 1.97–11.91; $p=0.001$), DQB1*0302 (OR: 4.58; 95% CI: 3.36–6.26; $p<0.0001$), DQA1*0301(OR: 3.02; 95% CI: 1.37–6.65; $p=0.0059$) and DQB1*0602 (OR: 0.19; 95% CI: 0.11–0.33; $p<0.0001$), DRB1*14 (OR: 0.18; 95% CI: 0.06–0.55; $p=0.0024$), and DQB1*0501 (OR: 0.47; 95% CI: 0.26–0.83; $p=0.0097$) were the most significant alleles associated with T1D. DRB1*0301-DQA1*0501-DQB1*0201 (OR: 13.50; 95% CI: 3.85–47.28; $p<0.0001$) and DRB1*1301-DQB1*0603 (OR: 0.25; 95% CI: 0.1–0.65; $p=0.004$) were the most significant risk and protective haplotypes associated, respectively. There were peptides binding to significantly HLA-DRB1 alleles and haplotypes found through the meta-analysis from islet cell protein tyrosine phosphatase and glutamic acid decarboxylase.
Conclusions: These results strengthen the effect of HLA-Class II on T1D in LA similar to Caucasians regardless of the latitudinal gradient and admixture. The shared chemical characteristics in critical pockets could explain the predisposition to present a "diabetogenic peptide" to T cells in this population.

Contents

1. Introduction

Type 1 diabetes mellitus (T1D) is a chronic disease most frequently presented in the childhood [1,2]. It is classified into type 1B (idiopathic) and 1A diabetes mellitus, which is mediated through

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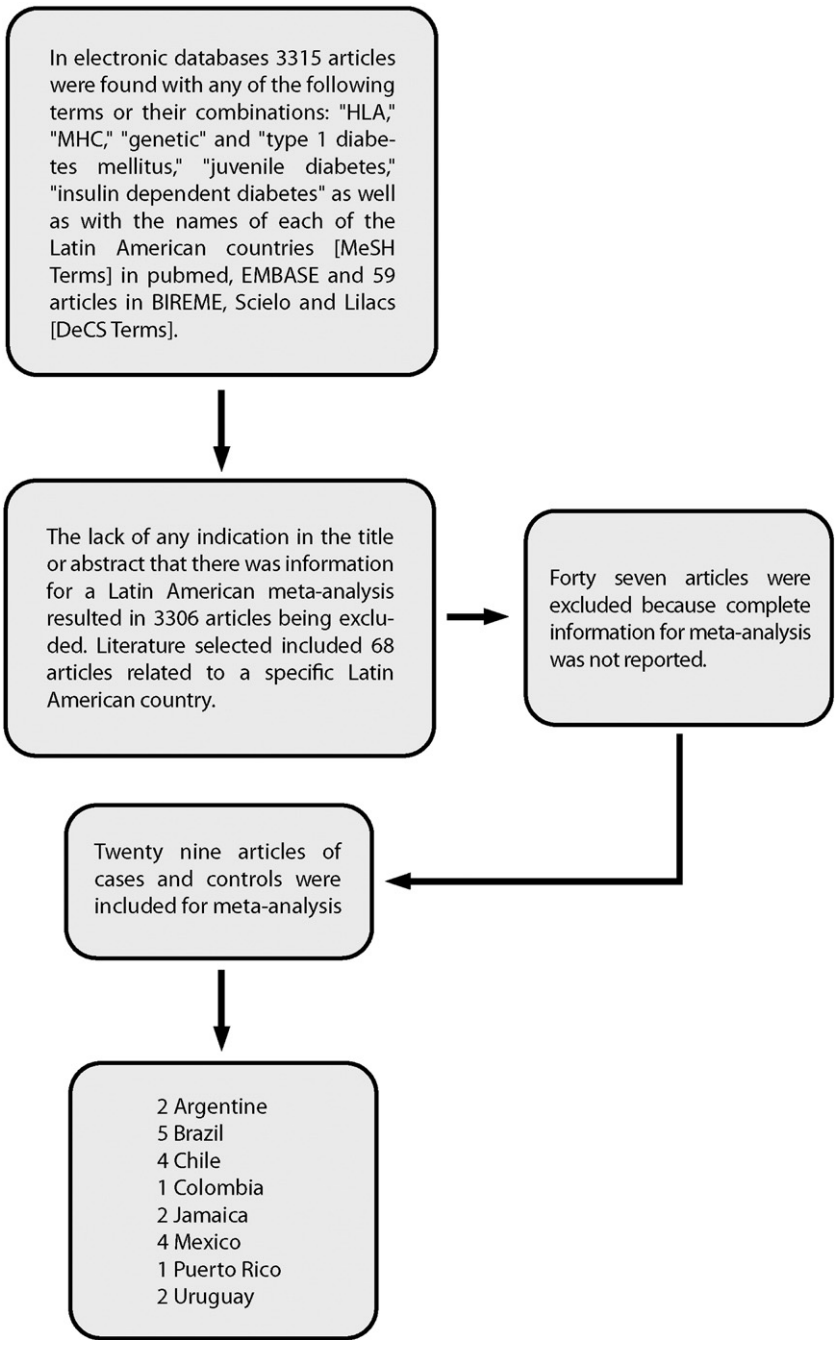


Fig. 1. Flowchart of included studies.

the immune system [3,4]. In T1D 1A, a genetically susceptible individual presents loss of tolerance to the pancreatic islet tissue triggered by environmental factors [5] and develops a progressive, immune-mediated destruction of pancreatic islet β cell, [3–6]. T1D is considered a multifactorial condition with complex interactions between genetic and environmental factors [1,3,7]. Its reported familial aggregation is close to 15 without any specific inheritance mode [3] but with an increased prevalence in the siblings of patients when compared to the general population [1,6]. First-degree relatives are more predisposed to developing T1D and to having a higher proportion of autoantibodies compared to the general population [8]. The concordance between monozygotic twins is between 30% and 50% [2] compared to the concordance between dizygotic twins which is <13% [2]. All of these arguments highlight the genetic component of the disease.

There are many genes involved in the development of T1D. Candidate gene studies carried out over a number of years identified four non-HLA T1D risk loci: INS, CTLA4, PTPN22 and IL2RA1 [9] but, results from linkage and association studies in T1D have long supported a model in which the major risk factor for T1D resides in the HLA region on chromosome 6p21 [1–3,9,10]. There is evidence showing that 40%–50% of the inherited susceptibility for the disease is contributed by HLA-DR–DQ [4]. Numerous studies have been done on the effect of the HLA-DR–DQ alleles, haplotypes, and genotypes on predisposition [11]. The HLA haplotypes that are the most frequently reported as involved in the susceptibility to T1D as risk factors are DRB1*0301-DQB1*0201, DRB1*0301-DQA1*0501-DQB1*0201, and DRB1*0401-DQB1*0302 [11]. The alleles that are the most frequently reported risk factors are DRB1*0301, DRB1*0401 [5], DPB1*0301[12] and DQA1*0301 [5]. At the same time, the DRB1*1501-DQB1*0602

Table 1
Individual characteristics of the studies included in the meta-analysis.

Study	Country			Risk alleles							
				DR alleles					DQ and DP alleles		
		Allelic frequency		DRB1*0301	DRB1*0405	DRB1*1201	DRB1*0402	DRB1*0401	DQA1*0301	DQA1*0501	DQB1*0302
		Cases	Controls	$\psi = 9.65$ Random	$\psi = 6.31$ Random	$\psi = 4.84$ Random	$\psi = 3.24$ Random	$\psi = 3.90$ Random	$\psi = 3.02$ Random	$\psi = 2.84$ Random	$\psi = 4.58$ Random
				$p = 0.0000$	$p = 0.0003$	$p = 0.001$	$p = 0.008$	$p = 0.0026$	$p = 0.0059$	$p = 0.002$	$p = 0.000$
				OR	OR	OR	OR	OR	OR	OR	OR
Caputo M et al.	Argentina	140	158	NA	NA	NA	NA	NA	NA	NA	8.44
Krochik AG et al.	Argentina	158	158	NA	NA	NA	NA	NA	NA	NA	516.11
Marques S et al.	Brazil	82	198	NA	NA	NA	NA	NA	NA	NA	NA
Hauache OM et al.	Brazil	252	150	NA	4.80	NA	4.00	9.25	NA	NA	NA
Fernandes A et al.[a]	Brazil	128	362	7.22	2.47	NA	2.18	1.94	NA	NA	4.92
Rassi DM et al., 2006[b]	Brazil	44	240	NA	NA	NA	NA	NA	NA	NA	NA
Rassi DM et al., 2006[c]	Brazil	12	12	1.00	0.31	NA	NA	9.21	NA	NA	1.00
Perez-Bravo F et al., 1995	Chile	126	148	NA	NA	NA	NA	NA	2.30	1.90	2.64
Perez-Bravo F et al., 1996	Chile	126	148	NA	NA	NA	NA	NA	2.30	1.90	2.64
Díaz N et al.[d]	Chile	114	250	NA	NA	NA	NA	NA	2.07	3.23	3.36
Perez-Bravo F et al., 1998	Chile	28	148	NA	NA	NA	NA	NA	0.57	7.08	1.94
Montoya F et al.	Colombia	52	112	22.28	130.97	5.27	1.33	1.78	NA	20.83	6.58
Heward JM et al.	Jamaica	72	158	NA	NA	NA	NA	NA	NA	NA	14.36
Mijovic CH et al.	Jamaica	74	164	NA	NA	NA	NA	NA	27.66	1.39	14.81
Erlich HA et al., 1996	Mexico	84	450	NA	NA	NA	NA	NA	NA	NA	NA
Erlich HA et al., 1993	Mexico	84	462	8.13	7.80	1.82	5.85	17.07	NA	NA	5.60
Gorodezky C et al.	Mexico	274	170	14.16	57.50	NA	28.11	NA	NA	0.94	3.98
Sanjeevi CB et al.	Mexico	70	78	NA	NA	NA	NA	NA	3.94	NA	4.15
Cruz TD et al.	Puerto Rico	182	164	NA	NA	NA	NA	NA	NA	NA	NA
Mimbacas A et al., 1998	Uruguay	30	30	NA	NA	NA	NA	NA	NA	NA	1.50
Mimbacas A et al., 2003	Uruguay	144	80	NA	NA	NA	NA	NA	NA	NA	6.38
Heterogeneity				*Q*	*Q*	*Q*	*Q*	*Q*	*Q*	*Q*	*Q*
				7.24	14.06	0.38	6.64	6.43	50.13	54.70	43.28
				p-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value
				0.124	0.015	0.530	0.156	0.169	0.000	0.000	0.000
				df	*df*	*df*	*df*	*df*	*df*	*df*	*df*
				4	5	1	4	4	5	6	15
				I^2	I^2	I^2	I^2	I^2	I^2	I^2	I^2
				44.8	64.4	0.0	39.8	37.8	90.0	89.0	65.3

NA: information not available from published data, Q: Cochran's test, I^2: Higgins' test, *df*: degree of freedom, ψ: OR.The OR from the random model is shown including studies with significant heterogeneity.
[a] 100 cases/128 controls for DQA.
[b] 35 cases/240controls for DQB.
[c] Microarrays 2006.
[d] 114 cases/242 controls for DQB.

haplotype is the most common protective factor [11]. The magnitude of their influence varies depending on the haplotype or allele carried [1,3,4,13].

Data from different parts of the world depict a latitudinal gradient in the incidence of T1D, meaning that rates could increase with distance from equator and inversely with ultraviolet radiation [1,14]. Latin America (LA) is considered a low T1D incidence area [2,5,15]. The ethnic composition of its population changes from one country to another [16] with a majority of them sharing a Spanish genetic background. This Spanish-Mestizo population ranges from 60 to 80% in Mexico, Colombia, Venezuela, Paraguay, Chile, Peru and Ecuador to less than 15% in Uruguay and Argentina [16]. In some Latin American areas, the native population is relatively unmixed [16]. All those facts speak of the rich and unique ethnic composition of the LA population. It is striking that in those unmixed, native populations there are few reported cases of T1D compared to the Chilean and Argentine Caucasian populations, which have a clear European background with a minimum of ethnic mixing and show an incidence of T1D that is similar to that reported in their ancestors' countries of origin [16].

Considering those factors and the lack of an individual study with the statistical power to determine the HLA related risk and protection alleles in the LA population that shares a unique genetic background which is the product of the racial mixing that has been occurring in our region as a consequence of migrations in the last 600 years, we examined the different HLA-Class II alleles and haplotypes identified as common contributors to T1D in studies done within our population through a systematic review of the literature followed by a meta-analysis.

2. Materials and methods

2.1. 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 October 2009. The search only included publications on HLA-Class II alleles and susceptibility to T1D in LA published in any of these three languages: Spanish, English or Portuguese. The search strategy used MeSH terms and the text words: "Type 1 Diabetes" [Major], "Autoimmune Diabetes" [Major] and HLA-DR/DQ antigens in combination with all LA countries, including Caribbean islands [MeSH]. For the search in the Spanish and Portuguese databases, the DeCS terms (Descriptores en Ciencia de la Salud): "Diabetes Mellitus Tipo 1," "Antígenos HLA" and "Complejo Mayor Histocompatibilidad" were used. No other criteria were taken into account.

The inclusion criteria were the following: 1) T1D diagnosis established using American Diabetes Association (ADA) or World Health Organization (WHO) criteria [17,18]; 2) If T1D diagnosis criteria

Risk alleles		Protective alleles								
DQ and DP alleles		DR alleles				DQ and DP alleles				
DQB1*0201	DPB1*0301	DRB1*11	DRB1*13	DRB1*14	DRB1*15	DQA1*0101	DQB1*0501	DQB1*0602	DQB1*0603	DPB1*0402
$\psi = 3.44$ Random	$\psi = 3.66$ Random	$\psi = 0.29$ Random	$\psi = 0.36$ Random	$\psi = 0.18$ Random	$\psi = 0.37$ Random	$\psi = 0.401$ Random	$\psi = 0.46$ Random	$\psi = 0.16$ Random	$\psi = 0.32$ Random	$\psi = 0.55$ Random
$p = 0.000$	$p = 0.0057$	$p = 0.000$	$p = 0.000$	$p = 0.0024$	$p = 0.0004$	$p = 0.051$	$p = 0.0097$	$p = 0.0000$	$p = 0.025$	$p = 0.0142$
OR	OR	OR	OR	OR	OR	OR	OR	OR	OR	OR
7.05	NA	NA	NA	NA	NA	NA	NA	0.18	NA	NA
309.12		NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	0.22	0.65	0.12	0.61	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	0.22	0.31	0.16	0.32	NA	NA	0.13	NA	NA
NA	NA	0.22	0.47	0.27	0.22	NA	NA	NA	NA	NA
3.57	NA	NA	NA	NA	NA	NA	NA	0.11	0.17	NA
NA	NA	NA	NA	NA	NA	0.25	0.45	0.15	NA	NA
2.05	NA	NA	NA	NA	NA	0.25	0.45	0.15	NA	NA
2.19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3.69	NA	NA	NA	NA	NA	0.22	0.30	0.16	NA	NA
6.19	NA	NA	NA	NA	NA	3.31	0.09	0.56	0.09	NA
0.41	NA	0.71	0.20	0.31	0.43	NA	1.79		0.10	NA
4.81	NA	NA	NA	NA	NA	0.21	0.31	0.12	1.11	NA
NA	5.86	NA	NA	NA	NA	NA	NA	NA	NA	0.44
4.88	NA	NA	NA	NA	NA	NA	1.36	0.36	0.92	NA
4.30	NA	NA	NA	NA	NA	0.39	0.37	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	2.29	NA	NA	NA	NA	NA	NA	NA	NA	0.71
2.98	NA	NA	NA	NA	NA	NA	NA	NA	0.17	NA
1.94	NA	NA	NA	NA	NA	NA	0.24	0.19	NA	NA
Q	*Q*	*Q*	*Q*	*Q*	*Q*	*Q*	*Q*	*Q*	*Q*	*Q*
47.07	3.57	3.94	2.34	0.30	1.37	42.61	31.65	3.72	7.49	1.42
p-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value	*p*-value
0.000	0.059	0.414	0.505	0.961	0.713	0.000	0.000	0.882	0.187	0.233
df	*df*	*df*	*df*	*df*	*df*	*df*	*df*	*df*	*df*	*df*
13	1	4	3	3	3	5	8	8	5	1
I^2	I^2	I^2	I^2	I^2	I^2	I^2	I^2	I^2	I^2	I^2
72.4	72.0	0.0	0.0	0.0	0.0	88.3	74.7	0.0	33.2	29.7

mentioned in numeral 1 were not used or another criteria were used, the article must mention the following: the patients were ketosis-prone having presented with either frank ketoacidosis or severe symptoms of short duration and were continuously dependent on insulin from diagnosis or serological evidence of an autoimmune pathologic process or evidence of pancreatic islet beta-cell destruction; 3) case–control design of the study; 4) use of molecular techniques to determine HLA polymorphisms; 5) publication of sufficient information to calculate Odds Ratios (OR); 6) being focused on a well defined LA population, and; 7) manuscript's publication in a peer-reviewed journal as a full paper. Summaries or abstracts were not accepted.

2.2. Data extraction

The following information was collected from each study: author, year of publication, a detailed description of ethnicity in the population studied, country of publication, HLA typing technique used, diagnosis criteria for T1D, 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 text.

2.3. 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 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% Confidence Interval (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. Moderator variables (categorical) were used for grouping by that variable, measuring the effect size in two groups of outcomes as well as comparing the summary for each group and summaries across all studies. The categorical moderator variables included were: population included, criteria used and technique applied. 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. Meta regression analysis was done to estimate the impact of continuous study moderators on overall heterogeneity (age of diagnosis).

2.4. Peptide-HLA alleles prediction

The IMGT/HLA Database (http://www.ebi.ac.uk/imgt/hla/) allele query form was used to search for the protein sequence for each allele significantly associated with T1D throughout the meta-analysis and then, the specific characteristics of the amino acids located in critical positions of each pocket (DQ/DR) were explored. Ethnic origins of each significantly associated allele were also described by the same database. The *homo sapiens* protein sequence of the main candidates for autoimmunity in T1D was sought by using PubMed protein tool (http://www.ncbi.nlm.nih.gov/protein/): islet cell protein tyrosine phosphatase (IA2) (AAH70053 950 aa), islet cell autoantigen (ICA) (NP_001129492 483 aa), Insulin (AAA59172 110 aa), islet cell cytoplasmic autoantigen (ICCA) (Q16849 979 aa) and glutamic acid decarboxylase (GAD) (CAA01913 594 aa). The results were used in a peptide format to develop a prediction model that identifies the peptides that are bound to HLA-DR alleles that were significantly associated with T1D throughout the meta-analysis by using artificial neural networks (http://www.cbs.dtu.dk/services/NetMHCIIpan/). Peptides were classified as strong binding (SB) peptides (threshold 50.000) and weak binding (WB) peptides (threshold 500.000). For the prediction of peptides that are bound to HLA-DQ alleles and haplotypes, the Immune Epitope Database Analysis Resource (IEDB) (http://tools.immuneepitope.org/analyze/html/mhc_II_binding.html) tool was used. In this computational model, peptides were classified as good binders according to the consensus prediction approach.

3. Results

3.1. Studies included

The initial search strategy allowed us to identify 68 studies (Fig. 1), which included review articles, for potential inclusion. Within this group, 29 association studies related to HLA-DRB1, HLA-DQA1 and/or HLA-DQB1 polymorphisms and susceptibility to T1D were identified. A total of 21 articles (1138 cases and 1920 controls) on the HLA-Class II region fit the selection criteria (Table 1). Detailed information on the articles included and reasons for excluding each of the 47 articles is given in supplementary Table 1 (http://www.urosario.edu.co/EMCS/Documentos/investigacion/crea/supplementary_material_T1DHLA2/). HW equilibrium data was only reported in 2 articles [19,20]. In two articles [12,21] the T1D diagnosis was based on the National Diabetes Data Group criteria [22]. Both met the inclusion criteria. One article [23] had both patients with defined, latent autoimmune adult diabetes (LADA) and patients with T1D WHO criteria (15 ± 9.2 years old) for HLA-DQB1 genotyping. The latter group was selected for meta-analysis.

3.2. HLA meta-analytic association

The meta-analysis of HLA-DP alleles was done based on data reported in 2 articles, HLA-DQA1 in 9 articles, HLA-DQB in 17 articles, HLA-DRB1 in 9 articles and haplotypes, 6 articles (see detailed references in supplementary material http://www.urosario.edu.co/EMCS/Documentos/investigacion/crea/supplementary_material_T1DHLA2/). Different types of alleles were significantly associated with T1D. These included ten high and three low resolution risk alleles as well as 5 high and 5 low resolution protective alleles. Detailed OR and *p*-value are found in Table 1 and Fig. 2. DRB1*0301-DQA1*0501-DQB1*0201 (OR: 13.50; 95% CI: 3.85–47.28; $p<0.0001$) and DRB1*1301-DQB1*0603 (OR: 0.25; 95% CI: 0.1–0.65; $p=0.004$) were the most significant risk and protective haplotypes associated respectively. There was no heterogeneity when comparisons variables were used and the impact of continuous study moderators (age at onset) on overall heterogeneity was not significant when using meta-regression (data not shown). Publication bias was not found (funnel plots and Egger's regression not shown).

3.3. Peptide, allele and haplotype HLA prediction

There were 10 SB peptides that bound to HLA-DRB1 risk alleles and 4 that bound to protective alleles all of which were significant results of the meta-analysis. Most of them were sequences of the IA2 protein that has been implicated in the antigen presentation process. Table 2 shows detailed information about the SB peptides including their affinity number and positions. The allele DRB1*1201 binds sequences from all five proteins included during the peptide prediction binding except insulin. IEDB analysis showed that the haplotype DQA1*0301-DQB1*0302 also binds sequences from three proteins (Table 2).

4. Discussion

This is the first meta-analysis searching for an association between HLA-Class II and T1D in LA population. The results showed that DRB1*0301, DRB1*0401, DRB1*0405, DRB1*1201, DQA1*0301, DQB1*0302 and DRB1*14, DQB1*0501, DQB1*0602 were the most significant risk and protective alleles associated with T1D respectively.

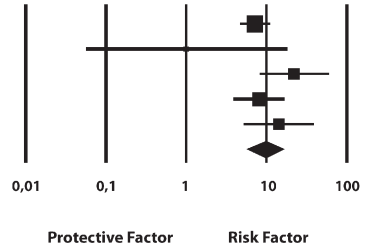
The haplotypes DRB1*03-DQA1*05-DQB1*02, DRB1*0301-DQA1*0501-DQB1*0201, and DRB1*0401-DQA1*0301-DQB1*0302 confer the greatest T1D susceptibility. In contrast, DRB1*01-DQB1*0501, DRB1*15-DQB1*0602, and DRB1*1301-DQB1*0603 provide the strongest protection.

The LA population is a mixed group with ancestries that include blacks, Caucasians and Amerindians, which reflects the notable racial, genetic and cultural diversity. The prevalence of T1D is less than world prevalence especially in LA countries which have an obvious Amerindian influence. This is important because previous results have suggested that in mestizo groups, the diabetogenic haplotype is from Mediterranean ancestry, while protection is from Amerindian genes [24].

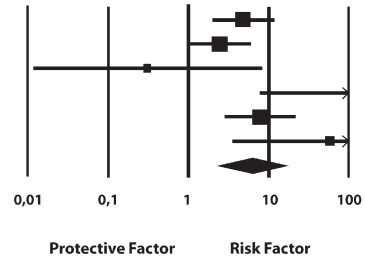
The effect of migration on the genetic background can be seen in the Chilean population in which the admixture of Spanish Caucasian genes with the native American genes could explain why there is a lower incidence of T1D when compared to Spain as well as by a lower frequency of DR3 and DQB1*0201 alleles in Chilean population [16]. Unlike Chile, Argentina has a primarily Caucasian population with a lower level of ethnic mixing. In this particular population, the incidence of T1D is comparable with the incidence in Caucasians [15,16].

This mixed group reveals different allele frequencies within racial groups. Comparisons across the T1D case populations revealed higher frequencies of DQA1*0301 in the low incidence Asian populations and LA countries compared to moderate–high incidence areas [25]. Although previous reports point to DQA1 loci as having a minimal effect on LA [26], this meta-analysis showed these loci to be a risk factor with an important OR. DQAI*0301 and DQAI*0501 have arginine (R) in position 52 while the DQBI*0201 and DQB1*0302 risk alleles contain sequence coding for an amino acid other than aspartic acid (D) in position 57 [alanine (A)] [25]. All of them were significantly associated with T1D in the present meta-analysis. These results show that the presence of R at position 52 of the DQα chain has been shown to cause increased risk for T1D in addition to the increased risk caused by the absence of D at position 57 of the DQβ chain [27]. These mutations of the strongly conserved D at position 57 of Class-II B-chain in HLA-DQ to another amino acid like A are associated with susceptibility to disease in LA as shown by this meta-analysis. This result is the same as those reported for other populations previously [11,28]. Some studies that have included analyses of crystallographic structures showed that the conserved salt bridge between β57 D and the invariant β76 R in class II MHC is broken when β57 D is mutated [29,30]. The interpretation of this mutation is that it opens the C-

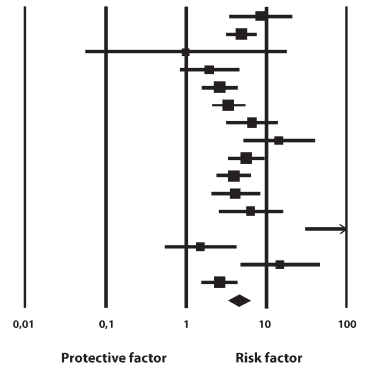
HLA Allele	Study name	Model	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
DRB1 *0301	Fernandes A et al		7,218	4, 627	11,260	8,711	0,000
	Rassi Dm et al microarrays		1,000	0,055	18,085	0,000	1.000
	Montoya F et al		22,275	8,294	59,823	6,157	0,000
	Erlich HA et al		8,127	3,907	16,904	5,608	0,000
	Gorodezki C et al		14,162	5,213	38,472	5,198	0,000
		Random	9,649	5,690	16,363	8,412	0,000



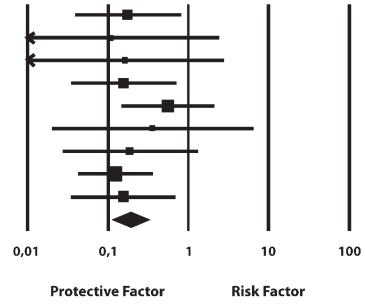
			Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
DRB1* 0405	Hauache OM et al		4,800	1,988	11,587	3,489	0,000
	Fernandes A et al		2,472	1,041	5,869	2,051	0,040
	Rassi DM et al microarrays		0,307	0,011	8,309	-0,702	0,483
	Montoya F et al		130,970	7, 702	2227,135	3,372	0,001
	Erlich HA et al		7,800	2,820	21,577	3,957	0,000
	Gorodezki C et al		57,500	3,510	941,954	2,840	0,005
		Random	6,314	2,308	17,278	3,588	0,000



			Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
DQB1* 0302	Caputo M et al		8,444	3,430	20,791	4,641	0,000
	Fernandes A et al		4,924	3,152	7,692	7,003	0,000
	Rassi DM et al microarrays		1,000	0,055	18,085	0,000	1,000
	Perez-Bravo F 1998 et al		1,941	0,834	4,518	1,539	0,124
	Perez-Bravo F 1995 et al		2,642	1,585	4,403	3,728	0,000
	Diaz N et al		3,362	2,096	5,390	5,032	0,000
	Montoya F et al		6,585	3,129	13,858	4,964	0,000
	Heward JM et al		14,363	5,183	39,801	5,124	0,000
	Erlich HA et al		5,603	3,314	9,475	6,430	0,000
	Gorodezki C et al		3,978	2,447	6,467	5,568	0,000
	Sanjeevi CB et al		4,153	2,061	8,368	3,983	0,000
	Mimbacas A et al		6,377	2,523	16,113	3,917	0,000
	Krochik A G et al		516,107	31,555	8441,285	4,381	0,000
	Mimbacas A 1998 et al		1,500	0,539	4,171	0,777	0,437
	Mijovic CH et al		14,815	4,849	45,267	4,730	0,000
	Perez-Bravo F 1996 et al		2,642	1,585	4,403	3,728	0,000
		Random	4,585	3,359	6,258	9,591	0,000



			Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
DQB1*0602	Caputo M et al		0,176	0,039	0,802	-2,245	0,025
	Rassi DM et al microarrays		0,109	0,005	2,365	-1,412	0,158
	Perez-Bravo F 1998 et al		0,163	0,009	2, 808	-1,249	0,211
	Perez-Bravo F 1995 et al		0,154	0,034	0,693	-2,439	0,015
	Montoya F et al		0,562	0,150	2,107	-0,854	0,393
	Erlich HA et al		0,359	0,020	6,352	-0,698	0,485
	Mimbacas A et al		0,187	0,027	1,304	-1,692	0,091
	Mijovic CH et al		0,123	0,043	0,355	-3,874	0,000
	Perez-Bravo F 1996 et al		0,154	0,034	0,693	-2,439	0,015
		Random	0,191	0,111	0,328	-5,989	0,000



			Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
DQB1*0603	Rassi DM et al microarrays		0,168	0,007	3,902	-1,112	0,266
	Montoya F et al		0,093	0,005	1,618	-1,630	0,103
	Heward JM et al		0,097	0,013	0,739	-2,252	0,024
	Erlich HA et al		0,916	0,109	7,704	-0,081	0,935
	Mimbacas A 1998 et al		0,167	0,033	0,853	-2,150	0,032
	Mijovic CH et al		1,144	0,325	3,823	0,172	0,863
		Random	0,323	0,120	0,871	-2,234	0,026

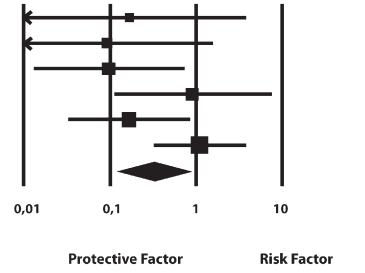


Fig. 2. Meta-analysis forest plot of T1D in Latin America. Meta-analysis forest plot for the most significant alleles associated with T1D. Each plot shows the effect size and precision for individual studies and for the combined effect calculated by the random model. Filled squares are proportional in size to study weights. Random shows Global OR for each allele.

Table 2
Predictive model results.

Protein	Allele or haplotype	DRB1*0401	DRB1*0405	DRB1*0301	DRB1*1201	DQA1*0301-DQB1*0302
IA2	PS	14HPSLSYEPALLQPYL	14HPSLSYEPALLQPYL	62TGTYILIDMVLNRMA	34HVHMSSGSFINISVV	955SKDQFEFALTAVAEE
	CP	LSYEPALLQ	LSYEPALLQ	ILIDMVLNR	VHMSSGSFI	FALTAVAEE
ICA	PS			62RTGTYILIDMVLNRM	34EHVHMSSGSFINISV	467SKDQFEFALTAVAEE
	CP			ILIDMVLNR	VHMSSGSFI	FALTAVAEE
ICCA	PS		11FIYIFTKISVDMYAG	19WPGVLFGMSIPSLWY	22KVLPLFIMVFPGMVS	
	CP		FIYIFTKIS	FGMSIPSLW	FIMVFPGMV	
GAD	PS				244AISNMYAMMI	202TNMFTYEIAPVFVLL
	CP				ISNMYAMMI	FTYEIAPVF

Strong binding peptides that are bound to HLA-DR risk alleles significantly associated with T1D throughout the meta-analysis are shown. For all alleles the NetMHCIIpan server was used. For the haplotype the Immune Epitope Database Analysis Resource (IEDB) was used. PS: peptide sequence; CP: core position; IA2: islet cell protein tyrosine phosphatase; ICA: islet cell autoantigen; ICCA: islet cell cytoplasmic autoantigen; GAD: glutamic acid decarboxylase.

terminus of the peptide binding groove, reduces the stability of class II MHC molecules and causes promiscuous peptide binding [30] which allows for recognition by the T-cell receptor.

HLA pocket 9 had been implicated with DR3, DR4, and DQ8 as being involved in susceptibility to T1D. The residual charge in pocket 9 of class II MHC DQ and DR underlies the strong linkage between the HLA and T1D. It is postulated [30] that the differences in polymorphic residues at P9, i.e. residual charge in a pocket, peptide-class II MHC binding affinity, kinetics and competition, are used as parameters for susceptibility or resistance to T1D. Alleles that confer susceptibility to disease are predicted to bind diabetogenic peptides strongly with slow dissociation kinetics. Two polar residues at β9 and β37 are proposed as the least common structural feature, which is the minimal set of polymorphism in DQ and DR molecules that predispose to T1D [30]. In the present meta-analysis, we found diverse alleles from the LA population that had that characteristic, among them DRB1*0405 and DQB1*0302, in addition to a non-D β57. It is hypothesized that the DR and DQ genes mediate this autoimmune disease by participating co-operatively and not working independently because they act in concert [31].

When we developed the computational model to predict alleles that bind GAD, IA2, ICA, ICCA and insulin peptides, interestingly DRB1*1201 was found to be the main allele that binds peptides from all the proteins included for the computational model excluding insulin. This was the fourth allele to have a significantly high association with T1D throughout the present meta-analysis and it has a known ethnic origin of Australian Aboriginal, Black, Caucasian and Oriental but has rarely been related to T1D [32] or to other autoimmune diseases [33]. Several specific proteins from beta-cells have been identified as targets of the autoimmune response in humans. One leading candidate autoantigen is the enzyme GAD65. The present computational model showed that among the peptides binding the haplotype DQA1*0301-DQB1*0302 there was a peptide from GAD65 202TNMFTYEIAPVFVLL216 that was strongly bound to this significant haplotype in LA. This peptide has been previously reported in models of transgenic NOD mice expressing HLA-DQ8 that were immunized with full-length, purified, recombinant GAD65. However, the peptide reported was the entire peptide from pos 201NTNMFTYEIAPVFVLLEYVT220. The above mentioned union gave the greatest, significant T-cell response in the mouse model [34]. A minimal sequence required for full stimulation of T-cell clones in response to a mixture of GAD65-derived peptides in T1D Japanese patients was determined to be the same sequence found in LA patients reported here—202TNMFTYEIAPVFVLL216 [35].

In conclusion, T1D is a disease that differs in prevalence and depends on the dominant ancestry of each country's population. In LA, its main prevalence is in populations with a mainly Caucasian ancestry. However, the alleles of the disease are similar around the world when we compare alleles to the peptides previously described as targets of autoimmunity in T1D. DR4 which binds sequences from IA2, has been widely described as one of the higher risk alleles in different autoimmune diseases including T1D. These results strengthen the effect of HLA-Class II on T1D in LA is similar to that in Caucasians regardless of the latitudinal gradient and admixture. Futures genotyping studies in which ancestry informative markers are included will allow us to better understand the origin of diabetogenic genes. The shared chemical characteristics in critical pockets could explain the predisposition to present a "diabetogenic peptide" to T cells in this population.

Take-home messages

- DRB1*0301, DRB1*0401, DRB1*0405, DRB1*1201, DQA1*0301, DQB1*0302 and DRB1*14, DQB1*0501, DQB1*0602 were the most significant risk and protective alleles associated with T1D respectively in LA.
- The alleles of the disease are similar around the world when comparing alleles to the peptides previously described as targets of autoimmunity in T1D.
- These results strengthen the effect of HLA-Class II on T1D in LA is similar to that in Caucasians regardless of the latitudinal gradient and admixture.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.autrev.2010.05.016.

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The common genes of autoimmunity: PTPN22 and CTLA4

In the pathogenesis of autoimmune diseases, genetic plays a major role. It has been demonstrated that several candidate genes located in common loci may be shared by different clinical conditions. HLA molecules have been associated with many of these disorders, but do not explain the whole genetic susceptibility for autoimmune diseases. Systemic lupus erythematosus, type 1 diabetes mellitus, multiple sclerosis and, at a lesser extent, rheumatoid arthritis show an increased familiar risk partly explained by genes in functionally key pathways. Due to the smaller populations, the genetic background of individuals affected with anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitis (AAV), namely Wegener's granulomatosis, microscopic polyangiitis and Churg-Strauss syndrome, has been less deeply explored. Most of the data come from small non-replicated studies and the only consistent show an association between AAV and HLA DBP1*0401. A number of polymorphisms in the PTPN22 and CTLA4 genes have been firmly associated in replicated studies with the abovementioned autoimmune diseases and they appear to be potentially implicated in the pathogenesis of AAV. Also, the rs2476601 of PTPN22 showed association with Wegener's granulomatosis in a study on 199 cases. Carr and colleagues recently performed an extensive analysis that confirmed the genetic association of AAV with PTPN22 and CTLA4 (Carr et al. Confirmation of the genetic association of CTLA4 and PTPN22 with ANCA-associated vasculitis. BMC Med Genet. 2009 1;10:121). The authors tested 11 candidate genes in 641 patients with AAV and 9115 controls, finding that rs247661 in PTPN22 and rs3087243 in CTLA4 were strongly associated with disease (P=1.4x10-4, O.R.=1.4; and P=6.4x10-3, O.R.=0.84, respectively). The role of these genes in the regulation of the immune response has been elucidated by several studies. PTPN22 is a central player as a negative regulator of T cell activation, and a similar role has been depicted for CTLA4. It is of interest that different polymorphisms in these genes may lead to the development of a variety of autoimmune diseases and that, conversely, the same polymorphism may be associated with either a protective or a predisposing effect with respect to a specific autoimmune condition. The pathways of autoimmunity are a fistful of molecules on a two pan balance where a distinct regulatory change is involved in different disease states. Due to the clinical importance of AAV and the low number of patients, the necessity of genome wide studies is deeply felt.

Topoisomerase I inhibitor in experimental lupus reverses proteinuria

Topoisomerase I inhibitor (irinotecan) was approved to treat colorectal cancer, and experimentally this drug prevented death of mice injected with foreign cytokines. In an interesting paper, Frese-Schaper *et al.* (**J Immunol 2010;184:2175-82**) have injected NZB x NZW F1 mice with irinotecan at 13 weeks of age and observed the animals until 90 weeks as prevention. The authors observed that all treated mice survived until the end of the study and did not develop high-grade proteinuria or nephritis compared to control group in which all animals had proteinuria by week 42. As treatment, in a groups of mice with grade 3 or 4 proteinuria, when irinotecan was infused, proteinuria was reduced, the disease entered into remission in 75% of the animals and survival increased. In conclusion, irinotecan seem to be promising as lupus treatment agent.