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HLA Risk Haplotype: Insulin Deficiency in Pediatric Type 1 Diabetes

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ABSTRACT

Background: Certain HLA class II haplotypes have long been related with the risk of developing type 1 diabetes. The presence of the HLA haplotype DRB1*04/DQA1*03/DQB1*03:02, together with specific β-cell autoantibodies, contributes to the development and/or severity of insulin deficiency in type 1 diabetes. **Objective**: To evaluate the association of HLA risk haplotype HLA-DRB1/-DQA1/-DQB1 with β-cell function and antibody markers in recent-onset type 1 diabetes patients, their siblings, and controls. **Methods**: We studied recently diagnosed type 1 diabetes pediatric patients, their siblings, and healthy controls, analyzing autoantibodies (anti-glutamic acid decarboxylase, anti-IA-2, and anti-insulin), HLA risk and protector haplotypes, and β-cell function (plasma proinsulin, insulin and C-peptide). X^2, ANOVA or Kruskal-Wallis and multiple logistic regression were used to analyze data. **Results:** We included 46 patients, 72 siblings, and 160 controls. Prevalence of anti-tyrosine phosphatase-related islet antigen 2 and anti-glutamic acid decarboxylase antibodies was higher in patients than siblings and controls. We found risk haplotype DRB1*04/DQA1*03/DQB1*03:02 in 95.7% of patients vs. 51.87% of controls; DRB1*03:01/DQA1*05/DQB1*02 in 47.8% of patients vs. 8.12% of controls; and DRB1*14/DQA1*05/DQB1*03:01 in 2.2% of patients vs. 20.0% of controls. With DRB1*04/DQA1*03/DQB1*03:02, the prevalence of antibodies was significantly higher in patients, although not within any single group. In regression model based on insulin secretion, only anti-tyrosine phosphatase-related islet antigen 2 antibodies and age were associated with the risk haplotype. **Conclusions:** The DRB1*04/DQA1*03/DQB1*03:02 haplotype increased the risk for lower insulin, proinsulin, and C-peptide concentrations, suggesting an association with the severity of insulin deficiency in type 1 diabetes patients. This haplotype, added to antibody positivity, is a predictor of deficient insulin secretion in a Mexican pediatric population. (REV INVES CLIN. 2016;68:128-36)

Key words: HLA. Proinsulin. C-peptide. Mexican. Antibody.

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INTRODUCTION

Type 1 diabetes (T1D) is a multifactorial and polygenic disease. It has long been recognized that class II alleles and haplotypes of the human leukocyte antigen system (HLA) on chromosome 6p21.3 are related to the risk of developing T1D[1]. There are many non-HLA T1D susceptibility genes, including: insulin gene (*INS*) on chromosome 11p15[2]; polymorphic, cytotoxic T-lymphocyte-associated protein gene (*CTLA4*) on chromosome 2q33[3]; protein tyrosine phosphatase, non-receptor type 22 gene (*PTPN22*) on chromosome 1p13[4]; interleukin-2 receptor alpha (*IL2RA*)[5]; and the interferon induced with helicase C domain (*IFIH1*) gene[6]. However, conditions and associations related with diabetes vary among different populations[7]. In addition, Rodríguez-Ventura, et al. found that risk haplotypes vary between type 1 and type 2 diabetes in the Mexican population[8]. As noted by Gómez-Díaz, et al.[9], various studies have found an association between incidence of T1D and the Latin American gene profile[10-12]. This was in line with the findings of the WHO DiaMond Molecular Epidemiology Sub-project performed in 1996[13]. Both Cruz-Tapias, et al.[14] and Gorodezky, et al.[15] have identified the risk alleles for T1D in the Latin American population, while Santos, et al.[16] and Mimbacas, et al.[17] reported risk alleles in Chilean and Uruguayan populations, respectively. However, Redondo, et al. identified DRB1*1401 and DQA1*0102/DQB1*0602 as protector alleles[18].

It has been accepted that positivity to β-cell autoantibodies is a risk factor for progression to T1D. As found in the European Nicotinamide Diabetes Intervention Trial (ENDIT), positivity for multiple antibodies in young patients is a fairly reliable indicator of later development of this disease[19]. This would be especially true in the case of patients' siblings with an HLA risk haplotype.

The aim of this study was to search for differences in markers of β-cell function (assessed by plasma proinsulin, insulin, C-peptide, HbA1c), β-cell antibodies (AB) (i.e. anti-glutamic acid decarboxylase 65 [GAD], anti-tyrosine phosphatase-related islet antigen 2 [IA-2], and anti-insulin), and lipid profile between individuals stratified according to their HLA Class II haplotypes (HLA-DRB1/-DQA1/-DQB1), in well-defined groups of recent-onset (≤ 3 months) T1D in Mexican pediatric patients, their siblings, and healthy controls.

METHODS

Study population

This was an analytical cross-sectional study of pediatric patients 2-17 years of age (cases), recently diagnosed (within the last three months) with T1D (according to the American Diabetes Association criteria)[20] and their siblings; healthy siblings included were free of acute infection at the time of inclusion. The control group consisted of healthy subjects of the same age and sex and their first-degree relatives (parents and siblings), enrolled among the children of employees of the Mexican Social Security Institute (IMSS) and from students of the elementary school "Escuela Benito Juárez", provided they were insured under IMSS, and were matched for age (± 6 months) and sex. Both they and their families were free of T1D and had no clinically evident infection. Patients with type 2 diabetes, maturity onset diabetes of the young, neonatal, or secondary diabetes, or any other autoimmune disease were excluded from the study. All the participants were of Mexican Mestizo origin, as defined by Gorodezky, et al.[21].

The study was conducted at the Endocrinology Department, Unidad Médica de Alta Especialidad (UMAE)-Hospital de Pediatría; Pediatric Endocrinology, UMAE Hospital General "Dr. Gaudencio González Garza", Centro Médico Nacional "La Raza"; and UMAE Hospital de Especialidades, Centro Médico Nacional (CMN) "Siglo XXI", all in Mexico City, with previous approval by the National Commission for Scientific Research from CMN SXXI, Instituto Mexicano del Seguro Social (Mexican Social Security Institute, IMSS).

All participants or their parents were asked to sign an informed consent. From all study subjects, a complete medical history (diabetes in the family, date of onset of diabetes, if applicable) and anthropometric measurements (age, weight, height, body mass index [BMI], blood pressure) were taken, and a 10 cc peripheral blood sample was obtained eight hours after the last meal for a complete blood count and blood chemistry.

Laboratory procedures

Complete blood count and blood chemistry were performed, including glucose, creatinine, total cholesterol, HDL-cholesterol and LDL-cholesterol, triglycerides, glycated hemoglobin (HbA1c), β-cell function tests

(plasma, proinsulin, insulin and C-peptide), and β-cell antibodies (anti-GAD, anti-IA-2, and anti-insulin). Class II-HLA typing (haplotype) was done. Anti-insulin antibodies in patients were determined only in those with T1D who had not yet started insulin therapy.

Anti-GAD and anti-IA-2 antibodies (AB) were determined by commercially available ELISA kits following the manufacturer's instructions (Genway Biotech, San Diego, CA). Anti-insulin-AB (detection range 0.312-20.0 U/ml) were also determined by commercially available ELISA kit following the manufacturer's instructions (Medipan GMBH, Berlin, Germany). Glucose, creatinine, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides were measured using the Synchron CX® analyzer (Beckman Systems, Fullerton, CA) according to standard protocols. The coefficient of variation for cholesterol and HDL-cholesterol were 3.3 and 2.5%, respectively. Insulin was determined by a quantitative radioimmunoassay (RIA) kit (Merck Millipore, St. Charles, Missouri), following manufacturer's instructions. Proinsulin and C-peptide concentrations were determined by RIA using reagents from Millipore Corporation (St. Charles, Missouri). The normal fasting proinsulin range was 7.9 ± 1.5 pM; sensitivity of the kit was estimated at 2 pM. The corresponding values for C-peptide were 0.16-4.99 and 0.021 nmol/l, respectively. The HbA1c percentage was determined from whole blood using ion exchange high-performance liquid chromatography (normal range 4-6%).

HLA-DR, -DQA, -DQB typing

Genomic DNA of T1D patients and their siblings was extracted from 1 ml peripheral blood mononuclear cells (PBMC), and was isolated using the QIAamp® DNA mini kit (Qiagen, Valencia, CA). DNA was quantified by spectrophotometry, adjusted to a final concentration of 60 ng/µl, and stored at −20 °C until tested. HLA typing for *HLA-DRB1* and *-DQB1* was performed according to the 11th International Histocompatibility Workshop[22], using a method designed to provide medium-low resolution of Class II type (SSP UniTray® Pel-freez Dynal Biotech, Roche, USA). Briefly, group-specific primer sets were used to amplify genomic DNA using a 96-well thermal tray. Genomic DNA sample was mixed with a reaction buffer and Taq polymerase. Mixture was dispensed to the 96 UniTray® wells for sealing and then thermal cycling. After completing 35 cycles, PCR products were loaded onto a 2% agarose gel for electrophoresis. Finally, the ethidium bromide stained gel was photographed and analyzed with UniMatchTM Plus analysis software program.

Statistical analysis

The Shapiro-Wilk test was used to assess if variables had a normal distribution. The X^2 test was used to compare proportions of haplotype frequencies. To compare characteristics among the groups, continuous variables were analyzed using either analysis of variance (ANOVA) test for normally distributed variables, or Kruskal-Wallis test for non-normally distributed continuous variables.

Multiple logistic regression analyses evaluating the association between the risk haplotype and antibodies and insulin secretion were performed using SPSS Windows v. 17.0 (SPSS, Inc). Haplotypes were obtained by maximum likelihood methods using Arlequin v. 3.0 software[23]. For all tests, $p < 0.05$ was considered significant.

RESULTS

We analyzed 46 patients, 72 siblings, and 160 healthy controls. Table 1 shows their anthropometrical, clinical, genetic, and immunological characteristics. As expected, all the anthropometrical characteristics were statistically different between the groups. Overweight and obesity were more frequent in the control group; in addition, they were older. There were statistically significant differences between groups in HbA1c, glucose, C-peptide, creatinine, total cholesterol, triglycerides, presence of anti-GAD-AB, and anti-IA-2-AB ($p < 0.001$), and HDL-cholesterol ($p = 0.019$).

When comparing positivity for antibodies between the three groups, the presence of one or more antibody was significantly more frequent in the patient group, followed by the siblings group ($p < 0.001$) (Table 1).

The analysis blocks of genetic frequency of HLA in patients and their siblings are shown in tables 2 and 3, respectively. There were significant differences in haplotype frequency between patients and controls: haplotype DRB1*04/DQA1*03/DQB1*03:02 was found in 95.7% of patients (n = 44) vs. 51.87% of controls (n = 83) ($p = 0.007$); and haplotype

Table 1. Anthropometric, clinical, genetic, and immunological characteristics of the study participants

	Patients with T1D	Controls	Siblings	p value
(n)	46	160	72	
Age, years	9.6 (7.8-12.9)	32.4 (24.2-42.0)	15.0 (9.1-23.0)	< **0.001**
Sex F/M (%)	25/21 (54.3)	86/74 (53.7)	36/36 (50.0)	< **0.001**
Weight, kg	36.6 ± 16.3 (10-68)	64.6 ± 20.2 (10-103)	53.6 ± 24.5 (14-112)	< **0.001**
Height, m	1.36 ± 0.21 (0.8-1.7)	1.56 ± 0.17 (0.8-1.8)	1.48 ± 0.21 (1.0-1.8)	< **0.001**
BMI, kg/m^2	18.4 ± 3.6 (11.39-25.69)	25.5 ± 5.3 (13.0-36.2)	22.6 ± 6.1 (12.9-41.9)	< **0.001**
Normal, n (%)	32 (69.6)	74 (46.2)	39 (54.1)	< **0.001**
Overweight, n (%)	13 (28.2)	55 (34.3)	24 (33.3)	< **0.001**
Obesity, n (%)	1 (2.2)	28 (17.5)	8 (11.1)	< **0.001**
SBP, mmHg	90 (80-110)	100 (69-130)	100 (80-130)	< **0.001**
DBP, mmHg	60 (50-70)	70 (60-80)	60 (40-100)	< **0.001**
HbA1c, %; mmol/mol	8.0 (6.6-9.2); 64 (49-77)	5.5 (5.0-5.8); 37 (31-40)	5.3 (4.9-5.6); 34 (30-38)	< **0.001**
Proinsulin, ng/ml	15.9 (7.7-21.4)	9.9 (6.7-16.3)	10.1 (7.3-14.7)	0.098
Insulin, µIU/ml pmol/l	16.2 (10.0-21.2) 97.19 (60-127.19)	15.6 (10.3-23.2) 93.6 (61.80-139.2)	13.9 (9.1-18.3) 83.40 (54.59-109.80)	0.530
C-peptide, ng/ml nmol/l	0.65 (0.23-1.26) 0.21 (0.07-0.41)	2.3 (1.5-3.0) 0.76 (0.49-0.99)	1.73 (1.10-2.52) 0.57 (0.36-0.83)	< **0.001**
Glucose, mg/dl	95 (80.0-127.5)	89 (83-97)	86 (80-90)	< **0.001**
C-peptide/glucose ratio	0.007 (0.002-0.011)	0.021 (0.014-0.031)	0.020 (0.012-0.028)	< **0.001**
Creatinine, mg/dl	0.52 ± 0.15 (0.2-1.0)	0.72 ± 0.18 (0.27-1.18)	0.63 ± 0.21 (0.23-1.1)	< **0.001**
Anti-GAD-AB, cases + (%)	16 (34.8)	20 (12.5)	13 (18.1)	< **0.001**
Anti-IA-2-AB, cases + (%)	20 (43.5)	13 (8.1)	7 (9.7)	< **0.001**
Anti-insulin-AB, cases + (%)	5 (10.9)	16 (10.0)	8(11.1)	0.799
≥ 1 antibody, n (%)	28 (60.9)	43 (26.9)	23 (31.9)	< **0.001**
≥ 2 antibodies, n (%)	12 (26.0)	7 (4.3)	5 (6.9)	< **0.001**
Total cholesterol, mg/dl	158.4 ± 40.0 (82-252)	179.2 ± 37.7 (93-278)	161.5 ± 36.3 (107-281)	< **0.001**
Triglycerides, mg/dl	66.5 (50.7-82.5)	124.0 (86.2-182.5)	91.0 (60.0-143.5)	< **0.001**
HDL-C, mg/dl	53.7 ± 14.0 (25.0-86.0)	47.1 ± 12.3 (13.0-79.0)	50.6 ± 13.4 (18.0-85.0)	**0.019**
LDL-C, mg/dl	141.9±35.3 (70.6-212.0)	145.8±38.6 (11.2-244.0)	138.8±33.1 (83.2-258.2)	0.155

Data are mean ± SD or median (interquartile range) for abnormal distribution. ANOVA or Kruskal Wallis was applied as appropriate. A p value < 0.05 was considered significant. Overweight was defined as BMI > 85 percentile and < 95 percentile in children and BMI > 25 and < 30 in adults; obesity as BMI ≥ 95 percentile in children and BMI ≥ 30 in adults. Note: In the patients with type 1 diabetes, anti-insulin Ab was only determined for those patients not on insulin therapy.
T1D: type 1 diabetes; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; GAD: glutamic acid decarboxylase; IA2: tyrosine-phosphatase antibodies; +: positive.

DRB1*03:01/DQA1*05/DQB1*02 in 47.8% (n = 22) vs. 8.12% (n = 13) (p = 0.002) (Table 4). Haplotype DRB1*14/DQA1*05/DQB1*03:01, thought to be protector, was present in 2.2% of patients (n = 1) vs. 20.0% of controls (n = 32) (p = 0.001) (Table 4).

Haplotypes DRB1*04/DQA1*03/DQB1*03:02 and DRB1*03:01/DQA1*05/DQB1*02, considered to indicate risk, were more frequent in patients than siblings or controls. These were also considerably more frequent in siblings than controls, as expected.

The most frequent risk haplotype (DRB1*04/DQA1*03/DQB1*03:02) was associated with a greater prevalence of positivity for one (p < 0.001) or ≥ two (p = 0.006) antibodies in the whole population. However, our sample size was not large enough to find differences between groups (Table 5).

Regarding β-cell function, the presence of the DRB1*04/DQA1*03/DQB1*03:02 haplotype was associated with a risk for higher proinsulin (p = 0.024) and lower C-peptide (p = 0.043) and C-peptide/glucose ratio

Table 2. Frequency of HLA-DRB1, DQA1, and DQB1 in patients with type 1 diabetes

HLA-DRB1	Patients		HLA-DQA1	Patients		HLA-DQB1	Patients	
Allele	n = 46	GF	Allele	n = 46	GF	Allele	n = 46	GF
DRB1*04	47	0.511	DQA1*03	49	0.533	DQB1*03:02	44	0.478
DRB1*03:01	22	0.239	DQA1*05	29	0.315	DQB1*02	27	0.293
DRB1*08	6	0.065	DQA1*01	6	0.065	DQB1*03:01	7	0.076
DRB1*12	4	0.043	DQA1*04	6	0.065	DQB1*04	7	0.076
DRB1*01	3	0.033	DQA1*02	2	0.022	DQB1*05	5	0.054
DRB1*13	2	0.022				DQB1*06	1	0.011
DRB1*07	2	0.022				DQB1*03:03	1	0.011
DRB1*14	2	0.022						
DRB1*09	2	0.022						
DRB1*01:03	1	0.011						
DRB1*16	1	0.011						

GF: genetic frequency.

Table 3. Frequency of HLA-DRB1, DQA1, and DQB1 in the siblings group

HLA-DRB1	Siblings		HLA-DQA1	Siblings		HLA-DQB1	Siblings	
Allele	n = 72	GF	Allele	n = 72	GF	Allele	n = 72	GF
DRB1*04	63	0.438	DQA1*03	67	0.465	DQB1*03:02	62	0.431
DRB1*03:01	16	0.111	DQA1*05	42	0.292	DQB1*02	28	0.194
DRB1*08	15	0.104	DQA1*04	15	0.104	DQB1*03:01	26	0.181
DRB1*14	9	0.062	DQA1*01	8	0.056	DQB1*04	15	0.104
DRB1*07	8	0.056	DQA1*02	8	0.056	DQB1*06	8	0.056
DRB1*12	8	0.056	DQA1*XX	4	0.028	DQB1*XX	3	0.021
DRB1*16	5	0.035				DQB1*03:03	1	0.007
DRB1*XX	4	0.028				DQB1*05	1	0.007
DRB1*09	4	0.028						
DRB1*11	4	0.028						
DRB1*15	3	0.021						
DRB1*13	3	0.021						
DRB1*01:03	1	0.007						
DRB1*01	1	0.007						

GF: genetic frequency.

($p < 0.001$) in patients (Table 5). No differences in the concentrations of insulin secretion indicators were observed in controls or siblings (Table 5). Siblings who were obese and carried the risk haplotype DRB1*04/DQA1*03/DQB1*03:02 were more likely to have positive antibodies than controls.

Multiple logistic regression models were performed, with proinsulin and C-peptide as dependent variables, to evaluate insulin secretion capacity as predictor of the risk haplotype DRB1*04/DQA1*03/DQB1*03:02 (Table 6). In the model based on proinsulin, only anti-IA-2-AB was associated (OR: 5.0; 95% CI: 1.125-22.691; $p = 0.035$). However, in the C-peptide model, only age was associated with the risk haplotype (OR: 1.0; 95% CI: 1.000-1.062; $p = 0.046$).

DISCUSSION

The presence of the HLA haplotype DRB1*04/DQA1*03/DQB1*03:02 together with antibody positivity contributes to the development and/or severity of insulin deficiency in T1D. The finding of the HLA risk haplotypes DRB1*04/DQA1*03/DQB1*03:02 and DRB1*03:01/DQA1*05/DQB1*02 was replicated in this sample of the Mexican population.

Table 4. Frequency of haplotypes in the study participants

Haplotype	Patients (n = 46)			Controls (n = 160)			Siblings (n = 72)		
	HF	(n)	Δ'	HF	(n)	Δ'	HF	(n)	Δ'
DRB1*04, DQA1*03, DQB1*03:02	**0.4783**	**44**	**1.0000**	**0.2600**	**83**	**1.0000**	**0.4236**	**61**	**0.9713***
DRB1*03:01, DQA1*05, DQB1*02	**0.2391**	**22**	**1.0000**	**0.0410**	**13**	**1.0000**	**0.1111**	**16**	**1.0000***
DRB1*08, DQA1*04, DQB1*04	0.0652	6	1.0000	0.1540	49	1.0000	0.1042	15	1.0000
DRB1*12, DQA1*05, DQB1*03:01	0.0435	4	1.0000	0.0000	0	–	0.0556	8	1.0000
DRB1*01, DQA1*01, DQB1*05	0.0326	3	1.0000	0.0660	21	1.0000	0.0069	1	1.0000
DRB1*04, DQA1*03, DQB1*02	0.0217	2	–0.8550	0.0000	0	–	0.0139	2	–0.8367
DRB1*07, DQA1*02, DQB1*02	0.0217	2	1.0000	0.0590	19	1.0000	0.048/6	7	0.8448
DRB1*01:03, DQA1*05, DQB1*03:01	0.0109	1	1.0000	0.0000	0	–	0.0069	1	1.0000
DRB1*14, DQA1*05, DQB1*03:01	0.0109	1	–0.7204	0.0000	0	–	0.0000	0	–
DRB1*04, DQA1*03, DQB1*04	0.0109	1	0.2923	0.0000	0	–	0.0000	0	0.4814
DRB1*09, DQA1*03, DQB1*02	0.0109	1	1.0000	0.0000	0	–	0.0208	3	0.6897
DRB1*09, DQA1*03, DQB1*03:03	0.0109	1	0.4713	0.0000	0	–	0.0069	1	1.0000
DRB1*13, DQA1*01, DQB1*05	0.0109	1	1.00000	0.0000	0	–	0.0000	0	–
DRB1*13, DQA1*01, DQB1*06	0.0109	1	1.0000	0.0302	10	1.0000	0.0208	3	1.0000
DRB1*14, DQA1*01, DQB1*05	0.0109	1	1.0000	0.0130	4	1.0000	0.0000	0	–
DRB1*14, DQA1*05, DQB1*03:01	**0.0109**	**1**	**1.0000**	**0.1010**	**32**	**1.0000**	**0.0625**	**9**	**1.0000***
DRB1*16, DQA1*05, DQB1*03:01	0.0000	0	–	0.0560	18	1.0000	0.0347	5	1.0000
DRB1*11, DQA1*01, DQB1*06	0.0000	0	–	0.0000	0	–	0.0069	1	1.0000
DRB1*11, DQA1*05, DQB1*03:01	0.0000	0	–	0.0000	0	–	0.0208	3	1.0000
DRB1*14, DQA1*05, DQB1*03:02	0.0000	0	–	0.0000	0	–	0.0070	1	–0.7097
DRB1*15, DQA1*01, DQB1*06	0.0000	0	–	0.0000	0	–	0.0208	3	1.0000

*A p value < 0.05 was considered significant.
HF: Haplotype frequency.

The DRB1*04 and DRB1*03:01 haplotypes were found to be strongly associated with the presence of T1D, and there seemed to be a protective effect of the DRB1*14 haplotype in this population, as found by Redondo, et al.[18] and Zuñiga, et al.[24], and also found in Chilean[25] and, recently, Iranian[26] populations. It is accepted that siblings of patients with diabetes share a greater risk of developing the disease than offspring of patients[27,28]. Aly, et al. indicated similar findings, especially when siblings share both risk haplotypes[29].

The excessive representation of native haplotypes could be the effect of at least one of two possible scenarios: (i) an increase in the presence of risk haplotypes for reasons as yet not understood, reducing the frequencies of the characteristic native haplotypes in the affected groups; (ii) the environment plays a role in the suppression of the autoimmune condition in such a way that native risk haplotypes do not trigger the autoimmune condition, similar to what occurs with other diseases such as lupus[30-32].

Our findings of risk and protector haplotypes differ from the literature, possibly due to the Mestizo combinations of the study population. Redondo, et al.[18] found protector trends among Caucasians in haplotypes (DQA1*0102/DQB1*0602, DRB1*1401/DQA1*0101/DQB1*0503) that never appeared in our population, neither in patients nor in controls. While we found the same most frequent risk haplotype as Gorodezky, et al. (DRB1*04/ DQA1*03/ DQB1*03:02)[15], the present study found this haplotype to be present at a much higher percentage (95.6 vs. 51.4% in patients, and 51.8 vs. 6.3% in controls). With regard to the risk haplotype DRB1*14/DQA1*05/DQB1*03:01, our findings are in agreement with Gorodezky, et al.[21]. However, when we analyzed the most common protector haplotype noted by Gorodezky, et al. (DRB1*0501/DQA1*0102/DQB1*0602) with positivity of antibodies, the protective value they reported was absent in our population. Further studies should target the protector value of these two haplotypes.

Table 5. Effect of the presence of risk haplotype DRB1*04/DQA1*03/DQB1*03:02 on antibody positivity and beta cell function in study participants

	Patients with T1D		Controls		Siblings		All groups
	HLA II risk		HLA II risk		HLA II risk		HLA II risk
	n (% of 44)	OR (95% CI) p	n (% of 83)	OR (95% CI) p	n (% of 61)	OR (95% CI) p	OR (95% CI) p
Anti-GAD-AB (cases +)	15 (34.0)	5.4 (0.6-48.2) 0.096	10 (12.0)	1.1 (0.4-2.9) 0.765	10 (16.3)	1.5 (0.3-6.4) 0.518	2.4 (1.209-5.015) **0.011**
Anti-IA-2-AB (cases +)	18 (40.9)	3.0 (0.5-16.8) 0.199	10 (12.0)	4.1 (1.1-15.8) **0.024**	6 (9.8)	1.3 (0.1-12.3) 0.785	3.8 (1.752-8.594) **0.001**
Anti-Insulin-AB (cases +)	4 (9.0)	1.0 (0.8-11.5) 1.000	5 (6.0)	0.5 (0.1-1.6) 0.259	5 (8.1)	2.4 (0.2-21.0) 0.401	0.9 (0.480-2.040) 0.977
≥ 1 positive antibodies	25 (56.8)	4.1 (0.8-19.5) 0.059	20 (24.0)	0.9 (0.4-1.9) 0.955	16 (26.2)	(0.3-3.2) 0.851	9.0 (2.560-31.638) **0.001**
≥ 2 positive antibodies	11 (25.0)	2.8 (0.3-20.2) 0.254	5 (6.0)	2.9 (0.5-15.7) 0.183	5 (8.1)	1.1 (1.0-1.2) 0.112	5.0 (1.4-17.9) **0.006**
Proinsulin (percentile > 75)	14 (31.8)	2.0 (1.3-2.8) **0.024**	12 (14.4)	1.3 (0.2-8.8) 0.727	7 (11.4)	0.5 (0.1-2.0) 0.356	1.5 (0.809-2.951) 0.186
Insulin (percentile > 75)	6 (13.6)	4.8 (0.5-45.4) 0.143	13 (15.6)	-0- 0.103	9 (14.7)	1.8 (0.440-7.787) 0.396	0.9 (0.478-1.849) 0.858
C-peptide (percentile > 75)	1 (2.2)	0.1 (0.008-1.32) **0.043**	12 (14.4)	0.2 (0.02-2.9) 0.272	7 (11.4)	1.4 (0.384-5.535) 0.578	0.2 (0.134-0.610) **0.001**
C peptide/ glucose ratio	---	---	11 (13.2)	0.8 (0.36-2.0) 0.735	10 (16.3)	2.0 (0.475-8.660) 0.334	0.4 (0.220-1.094) 0.079

NOTE: In the patients with type 1 diabetes, anti-insulin-AB was only determined for those patients not on insulin therapy. Abnormal values for proinsulin, insulin, and C-peptide were those above the 75 percentile. There were no cases of positivity for any antibody in the presence of HLA II protector haplotypes.
The X^2 test was used to compare proportions of haplotype frequencies. A p value <0.05 was considered significant.
T1D: type 1 diabetes; +: positive; IA2: tyrosine-phosphatase antibodies; GAD: glutamic acid decarboxylase.

Table 6. Association between the HLA haplotype DRB1*04/DQA1*03/DQB1*03:02 and antibodies and insulin secretion

Model 1. Proinsulin (percentile > 75)

Variable	Beta	OR	95% CI	p value
Anti-IA-2	1.620	5.052	1.125-22.691	0.035

Model 2. C-peptide (percentile > 75)

Variable	Beta	OR	95% CI	p value
Age	0.030	1.031	1.000-1.062	0.046

Models adjusted for age, sex, anti-GAD, anti-IA 2, anti-insulin, LDL-cholesterol and triglycerides, according to risk haplotype DRB1*04/DQA1*03/DQB1*03:02.
IA2: tyrosine-phosphatase antibodies.

Patients carrying at least one risk haplotype were younger at the age of onset than those not carrying any risk haplotype, as reported by Easton[33]. In addition, according to Gillespie, et al.[34], younger age of the patient at onset of the disease increases the risk for siblings.

Patients had significantly higher concentrations of proinsulin and lower concentrations of C-peptide than controls or siblings. These differences in insulin, proinsulin, and C-peptide concentrations may be explained by the fact that genetic load impacts the progression of the disease. The difference in antibody titers among the patient group shows the role of HLA association with an immune condition rather than a metabolic explanation of the disease.

Lower insulin, proinsulin, and C-peptide concentrations in carriers of two risk haplotypes may be taken as evidence of genetic factors underlying the severity of insulin deficiency in T1D. Genetic testing in siblings may be of clinical interest when a child is diagnosed with T1D, and these children should be carefully monitored. Further studies need to be conducted in populations with different ethnicities and distinct ancestral genetic proportions.

Evidence has shown that age at onset of diabetes also affects anti-GAD-AB and anti-IA-2-AB positivity. Howson, et al. found that both were associated with older age at diagnosis, while the same study indicated a

faster decline in antibody positivity with younger age at onset of the disease[35]. Anti-GAD and anti-IA-2 antibodies are among the most reliable markers of autoimmune activity in diabetes in general[36]. Nevertheless, the percentages of positivity relate with several factors, including ethnicity, testing method, and duration of the disease. In the present study, patients with T1D showed a higher percentage of positivity for anti-GAD and anti-IA-2 antibodies than healthy subjects, as expected. In the presence of risk haplotypes, it would be expected that the frequency of autoimmune antibodies would increase in all subjects. However, siblings carrying the risk haplotype DRB1*04/DQA1*03/DQB1*03:02 showed a higher percentage of positivity for antibodies than controls carrying this haplotype, supporting the need for careful monitoring.

In the regression analysis models, only anti-IA-2-AB was significant when using a model based on proinsulin in carriers of haplotype DRB1*04/DQA1*03/DQB1*03:02. Age was significant in the C-peptide model. This is in contrast with the findings of the ENDIT study[19], which showed that antibody positivity and age determined risk, but genotype did not.

The present study has some limitations. First, the sample was relatively small, and further studies with larger samples and follow-up are suggested. In addition, the ages and BMI of the control group were different from those of the cases. These differences were controlled in the multivariate analyses.

In conclusion, the presence of the DRB1*04/DQA1*03/DQB1*03:02 haplotype increased the risk for lower insulin, proinsulin, and C-peptide concentrations, suggesting an association with the severity of insulin deficiency in type 1 diabetes patients. This haplotype, added to β-cell antibody positivity, is a predictor of deficient insulin secretion in the Mexican pediatric population.

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