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Full Length Research Paper

Human leukocyte antigen (HLA) polymorphism and type 1 diabetes in the Moroccan population

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Insulin-dependent diabetes mellitus or type 1 diabetes is an autoimmune multifactorial disease which has a great socio-economic impact. In Morocco, less is known about the contribution of Human leukocyte antigen (HLA) alleles to type 1 diabetes susceptibility. Our study focused on evaluating the distribution of class II HLA genes among Moroccan patients presenting type 1 diabetes coming from different regions of the country. A total of 76 patients diagnosed with type I diabetes were compared to a group of 248 healthy controls matched by age, sex, and ethnic origin. The HLA class II (DR and DQ) typing of patients and controls was performed using polymerase chain reaction sequence-specific primers (PCR-SSP). Ambiguous typings were reanalyzed by Luminex technology. We found a significant association between HLA-DRB1*03 (p = 0.0001), DRB1*04 (p = 0.0001), DQB1*02 (p = 0.0001, OR = 4.4) and DQB1*03 (p = 0.002) alleles frequencies and the susceptibility to diabetes. The HLA-DQB1*06 allele was significantly decreased in patients (p = 0.008). DRB1*03-DQB1*02, DRB1*04-DQB1*03 and DRB1*04-DQB1*02 haplotypes induce susceptibility to type 1 diabetes while DRB1*13-DQB1*06 and DRB1*15-DQB1*06 haplotypes seems to confer a protective effect in our population. These data highlights that Moroccans diabetic patients do share a series of traits with diabetics of other origins but also have specific characteristics.

Key words: Type 1 diabetes, susceptibility, human leukocyte antigen (HLA), Moroccan population.

INTRODUCTION

Insulin-dependent diabetes mellitus or type 1 diabetes (T1D) is the third most common disorder of childhood, affecting between 1 and 4 in 10 000 individuals before the age of 30 with an incidence rate that appears to be increasing in all studied countries (Balafrej, 2003; Behrooz- Alizadeh et al., 2008; Patterson et al., 2009).

T1D is a multifactorial autoimmune disease resulting from the destruction of insulin-producing beta cells in the pancreas by autoreactive T cells. Such destruction results in clinically insufficient insulin production and in a dysregulation of glucose homeostasis (Atkinson and Maclaren, 1994). Symptoms accompanying the disease include high level of glucose in patient's blood and urine, T cell infiltration into the pancreas and the presence of autoantibody against insulin and beta cell antigens (Parry and Brooks, 2008).

Genetically, the occurrence of diabetes mellitus depends on several genes and mainly on the one situated at the level of the major histo-compatibility complex (MHC) (Behrooz- Alizadeh et al., 2008). Cross-ethnic studies have now confirmed that three Human Leucocytes Antigen (HLA) class II genes, DRB1, DQA1 and DQB1, contribute directly to T1D (Abid Kamoun et al., 2002; Nepom and Erlich, 1991; Parry and Brooks, 2008).

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The role of HLA in T1D was first indicated by associations in Caucasians with the HLA-DR3 and -DR4 antigens encoded at the DRB1 locus. Subsequently, DQB1 and DQA1 genes were shown to be more strongly associated with T1D, and the DQ molecule was considered as the primary susceptibility factor. Several studies have clearly demonstrated that both DQ and DR molecules influence T1D susceptibility (Park et al., 1998). A protective effect of HLA class II molecules was also demonstrated. Transracial studies have reported that the alleles involved in T1D appear to be different in various populations (Aribi et al., 2004; Chen et al., 1999; Platz et al., 1981; Sheely, 1992; Thomson et al., 1988; Valdheim et al., 1989).

In Morocco, very few investigations have been undertaken to study the impact of genetic background on the risk to develop T1D when the average prevalence is about 2% (Belkhadir and El Alaoui, 1993). Until now, available studies have been conducted in isolated populations from particular origins like Berber and Jewry (Izaabel et al., 1996; Witt and al., 1994), whereas the present population comprises different ethnic groups (Arabic, Berber and African). Morbidity and mortality are increased because of late diagnosis and lack of regular checks.

The aim of this study was to investigate the relationship between T1D and HLA in a sample of Moroccan population, from diverse regions of the country, and to determine the predisposing and the protective alleles to the disease.

MATERIALS AND METHODS

Patients and controls

A total of 76 patients consecutively diagnosed (before fifteen years old) with T1D were recruited from the department of endocrinology of Ibn Sina University Hospital of Rabat during the period 2005 to 2011.

The sample consisted of 37 women and 39 men between 2 and 64 years (mean age 21 years). The patients were compared with 248 healthy unrelated control subjects and matched by age, sex and ethnic origin. The mean age of the control population was 29 years and the sex ratio (males/females) was 127/ 121. These controls were either volunteers as bone marrow- or kidney-donors or just volunteers taking part in the study.

The populations compared in the present study were from diverse regions of Morocco.

Human leukocyte antigen (HLA) typing

Blood samples were collected and analyzed at the Ibn Sina University Hospital of Rabat and analyzed in the laboratory of Immunology of this hospital.

DNA was first extracted from the buffy coat fraction of blood samples using a commercial kit (Qiagen). The HLA typing of the HLA class II alleles (-DRB1 and –DQB1) was performed by polymerase chain reaction-sequence specific primers (PCR-SSP) technology using micro generic HLA DNA typing trays (One Lambda) in accordance to the manufacturer's protocol. Ambiguous typings (blanks, not clearly identified alleles) were reanalyzed by Luminex technology polymerase chain reaction sequence-specific oligonucleotide (PCR-SSO).

Statistical analysis

Allele, genotype and haplotype frequencies, p values, odds ratio (OR) and 95% confidence interval (CI) were calculated using the commercial SPSS software package for Windows, version 10.0. The frequencies in patients and controls were compared with Fisher exact test. A p value less than 0.05 was considered statistically significant. Corrected p values (pcorr) were calculated using the Bonferoni inequality method. In all cases, pcorr refers to corrected p values which were obtained by multiplying crude p values by the number of comparisons (Millot, 2009).

RESULTS

The HLA-DRB1 and –DQB1 allele frequencies observed in the Moroccan patients with T1D and healthy controls are presented in Table 1. The most frequent alleles identified in the controls were HLA-DRB1*03, -04, -07, -15, -13 and HLA-DQB1*02, -03, -06. Some alleles were scarce (HLA- DRB1*12 and DRB1*09 and HLA-DRB1*16.) When controls and patients were compared, HLA-DRB1*03 and -DRB1*04 alleles frequencies were increased significantly in patients ($p < 0.0001$). On the other hand, the frequencies of HLA -DRB1*15 decreased in the patient group (pcorr almost significant).

The distribution of the DQB1 alleles reveals that the frequencies of DQB1*02 and DQB1*03 in T1D patients were significantly higher than that in the control group with a respective Odds ratio of 4.4 ($p < 0.0001$) and 2.4 ($p = 0.002$). Conversely, the frequency of the HLA-DQB1*06 allele was significantly decreased in the controls compared to diabetes patients ($p < 0.0001$) and DQB1*05 frequency was also reduced by half in patients.

Table 2 presents the different combinations of DRB1*03, DRB1*04, DRB1*13 and DRB1*15 alleles observed in patients and healthy controls. No significant differences in the frequencies was observed in patients except for DRB1*03/DRB1*04 genotype ($p < 0.0001$, OR = 7.9). To evaluate the risk associated with the different combinations of DQB1, three DQB1 alleles were considered: DQ1*02, DQB1*03 and DQB1*06 (Table 3). DQB1*02/DQB1*03 was really significantly associated with T1D (OR = 4.9, $p < 0.0001$). whereas DQB1*02/*06 and DQB1*03/*06 were more observed in healthy controls than in patients ($p=0.009$ and $p=0.014$ respectively).

HLA DRB1*-DQB1* haplotypes frequencies were calculated for diabetic patients and healthy controls. The most frequent combinations are illustrated in Table 4. Three haplotypes frequencies in T1D patients were significantly higher in comparison to the control group: DRB1*03-DQB1*02 ($p < 0.0001$), DRB1*04-DQB1*03 ($p < 0.0001$). The highest risk for type 1 diabetes seemed to be attributed to DRB1*04-DQB1*02 (OR = 6, $p < 0.0001$). Otherwise, the frequency of DRB1*13-DQB1*06 and DRB1*15-DQB1*06 haplotypes were diminished in patients

Table 1. HLA-DRB1 and allele frequencies in Moroccan type 1 diabetes patients and healthy controls.

Allele	Control		Patient		p/pcorr	OR	CI
	n = 248	AF (%)	N = 76	AF (%)			
DRB1*	2n		2n				
1	39	7.9	7	4.6	0.2		
3	85	17.2	47	3.9	<0.0001/0.001	3.1	1.8 - 5.3
4	87	17.6	48	31.6	<0.0001/0.001	3.2	1.9 - 5.4
7	73	14.7	17	11.2	0.2		
8	22	4.5	5	3.3	0.6		
9	3	0.6	2	1.3	0.3		
10	15	3.0	1	0.7	0.1		
11	37	7.5	5	3.3	0.1		
12	2	0.4	1	0.7	0.6		
13	49	9.9	5	3.3	0.008/0.1	0.3	0.1 – 0.8
14	11	2.2	1	0.7	0.3		
15	53	10.7	6	4.0	0.006/0.08	0.3	0.1 – 0.8
16	2	0.4	2	1.3	0.2		
Blank	18	3.7	5	3.3	1		
DQB1*							
2	149	30.1	66	4.4	<0.0001/0.0006	4.4	2.2 – 8.9
3	134	27.0	56	36.9	0.002/0.01	2.8	1.4 – 4.2
4	24	4.9	3	1.9	0.15		
5	68	13.7	10	6.6	0.014/0.08	0.4	0.2 – 0.8
6	99	20.0	9	5.9	<0.0001/0.0006	0.2	0.1 – 0.4
Blank	22	4.5	8	5.3	0.82		

AF = Allele frequency; pcorr = P value corrected for the number of comparisons; OR = odds ratio (only statistically significant OR are presented) CI = confidence interval.

Table 2. The DRB1* genotypes of type 1diabetes patients and healthy controls.

DRB1* genotype	Control		Patient		p	OR	CI
	n = 248	(%)	N = 76	(%)			
DR3/DR13	14	5.6	1	1.3	0.201		
DR3/DR15	15	6	2	2.6	0.378		
DR4/DR13	9	3.6	-	-	-		
DR4/DR15	7	2.8	1	1.3	0.686		
DR3 / DR4	13	5.2	23	30	<0.0001	7.9	3.7 – 16.5
DR13/DR15	4	1.6	2	2.6	0.628		
DR3/DR3	-	-	4	5.3	-		
DR4/DR4	3	1.2	4	5.3	0.055		
Others genotypes	183	74	39	52.6			

OR, Odds ratio (only statistically significant OR are presented) ; CI, confidence interval

with respective p values of 0.006 and 0.005.

DISCUSSION

Morocco is a Maghrebian country situated in North Africa, between the Mediterranean world, the middle and near east and Black Africa. Thanks to its strategic location, Morocco has been a “converging meeting land” since antiquity. The present Moroccan population comprises different ethnic groups (Arabic, Berber, Africa) with a strong influence of various immigrations, mainly

Table 3. The DQB1*genotypes of type 1diabetes patients and healthy controls.

DQB1* genotype	Control		Patient		p	OR	CI
	N = 248	(%)	N = 76	(%)			
DQ2/DQ3	37	14.9	35	46.1	<0.0001	4.9	2.8 - 8,6
DQ2/DQ6	35	14.1	3	3.9	0.014	0.3	0.1 - 0,8
DQ3/DQ6	32	12.9	2	2.6	0.009	0.2	0.1 - 0.8
DQ2/DQ5	24	9.7	4	5.3	0.35		
DQ3/DQ5	22	8.9	2	2.6	0.08		
DQ2/DQ2	17	6.9	9	11.8	0.225		
DQ5/DQ6	14	5.6	1	1.3	0.2		
DQ3/DQ3	12	4.8	7	9.2	0.167		
Others genotypes	55	22.2	13	17.2			

OR, Odds ratio ; CI, confidence interval.

Table 4. Most frequent HLA DR-DQ haplotypes in type 1 diabetes patients and healthy controls.

HLA-DR-DQ haplotype	Control	Patient	P	OR	CI
	HF (%)	HF (%)			
DR3-DQ2	7.8	15.1	<0.0001	3.4	2.0 - 5.8
DR4-DQ3	7.8	15.1	<0.0001	3.4	2.0 - 5.8
DR4-DQ2	2.7	10.5	<0.0001	6.0	3.2 - 10.9
DR7-DQ2	6.9	5.6	0.5		
DR7-DQ3	2.3	2.6	0.8		
DR1-DQ5	3.4	2.0	0.2		
DR11-DQ3	3.4	1.7	0.109		
DR15-DQ6	5.2	1.7	0.005	0.3	0.1 - 0.7
DR13-DQ3	2.2	1.0	0.22		
DR13-DQ6	3.3	0.7	0.006	0.2	0 - 0.8

HF, Haplotype frequencies ; OR , odds ratio (only statistically significant OR are presented) ; CI , confidence interval.

Mediterranean. All these populations probably contributed to the Moroccans genetic pool (Canosi et al., 2010)

The HLA alleles and haplotypes distribution among Moroccan T1D population is not well defined because it has not been extensively studied. In this report, we analysed HLA class II polymorphism in Moroccan patients affected by T1D from diverse region of Morocco and we compared them to healthy controls of the same ethnic origin.

Distribution of alleles and haplotypes observed in our controls are in accordance with previous Moroccan studies (Brick et al., 2006; Oumhani et al., 2002; Canosi at al., 2010). Our data confirm the well-known positive association between T1D and HLA-DRB1*03 and -DRB*04. Furthermore, the high frequency of the heterozygous combination DRB1*03/DRB1*04 in patients (30.3%) indicates a synergistic effect on the risk to develop the disease. Indeed, reviews carried out during the last years revealed that 80 to 95% of the Caucasian insulin–dependent diabetics belong to the group DRB1*03 and/or DRB*04 against 40 to 50% from the control group (Abid Kamoun et al., 2002; Atkinson and Maclaren, 1994; Bouhours-Nouet and Coutant, 2005; Chen et al., 1999; Thomson, 2001). It is interesting to note that the DRB1*04 frequency observed in our study was significantly associated to type 1 diabetes ($p < 0.0001$) even though this allele was highly represented in the Moroccan healthy population.

The associations characterizing Caucasian populations (DR3, DRB4) have neither been found in Africans whose susceptibility to T1D is associated with DR7 and DR9, nor was it found among Asian populations (DR4 and DR9) (Danze et al., 1997). HLA-DRB1*09 was low in both healthy controls and patients. This allele is very rare in North African populations but its association with T1D susceptibility has previously been reported in Moroccan diabetics all originating from the Souss region and mostly of Berber origin (Izaabel et al., 1996). In contrast, DRB1*09 is associated with the disease in Japanese and Korean and the haplotype DRB1*09/DRB1*09 is a very high risk genotype in Asian populations (Ikegami et all 2007; Kanga et al., 2004; Park et al., 1998). The

differences among alleles associated with T1D in Moroccan and Asian populations can be explained by the difference in the presence or absence of alleles in each population (Aribi et al., 2004).

Concerning DQB1 alleles, the present study shows a significant association of DQB1*02 (OR = 4.39, $p < 0.0001$) and DQB1*03 (OR= 2.4, $p = 0.002$) with T1D. This association was described in several groups but not in Japanese people (Abid Kamoun et al., 2002; Chen et al., 1999; Caillat-Zucman, 2001; Parry and Brooks, 2008).

The comparison of the allele frequencies in Moroccan T1D and controls revealed a protective role for DQB1*06, DRB1*15 and DQB1*05 (to lesser degree) since their frequency were higher in the controls than in the patients. A similar observation had already been reported (Bouhours-Nouet and Coutant, 2005; Chen et al., 1999; Caillat-Zucman, 2001).

In contrast to Tunisian, Greek, Japanese, Polish and French populations, DRB1*11 allele was not protective in Moroccan T1D patients (Abid Kamoun et al., 2002; Krokowski et al., 1998; Zung et al., 2004).

Interestingly, our study revealed that the presence of DQB1*06 on DQB1*02/DQB1*06 and DQB1*03/DQB1*06 genotypes has a protective effect, even in the presence of the predisposing DQB1*02 and DQB1*03 alleles. This confirms the finding of previous studies (Parry and Brooks, 2008) in which DQB1*06 conferred a strong dominant protection even when combined with alleles with high risk.

Studies conducted on different populations showed that several HLA-DRB1–DQB1 haplotypes are associated with a spectrum of different disease risks, ranging from strong susceptibility to almost complete protection (Behrooz Alizadeh and Bobby, 2008). Our results shows that the haplotypes DRB1*04-DQB1*03 and DRB1*03-DQB1*02 increase the susceptibility to T1D while the haplotype DRB1*15-DQB1*06 protect against the disease. These results were close to those reported in most Caucasian, Asian and Black populations (Abid Kamoun et al., 2002; Behrooz- Alizadeh and Bobby Koeleman, 2008; Park et al., 1998). In our series, DRB1*13-DQB1*06 haplotype protects against the disease and seems to be a feature of the Moroccan population. The protective effect of the DRB1*11-DQB1*03 haplotype was not shown in our population contrasting with Tunisian, French, Greek and Japanese populations (Abid Kamoun et al., 2002).

It would be interesting to confirm the study by increasing the sample sizes. Moreover, these results may serve as a reference for future studies to identify specific alleles of predisposition to the disease in our population.

REFERENCES

Abid Kamoun H, Hmida S, Kaabi H, Abid A, Slimane Houissa H, Maamar M (2002). HLA polymorphism in type 1 diabetes Tunisians. Ann. Génét. 45:45-50.

Aribi M, Moulessehoul S, Benabadji AB, Kendoucitani M (2004). HLA DR phenotypic frequencies and genetic risk of Type I diabetes in west region of Algeria, Tlemcen. BMC Genet. 5:24-30.

Atkinson MA, Maclaren NK (1994). The pathogenesis of insulin dependent diabetes mellitus. N. Engl. J. Med. 331:1428-1436.

Balafrej A (2003). Management and treatment of diabetic children at the University Hospital Centre in Rabat: an example of partnership or personal initiative on the periphery of the School of Medicine. Santé publique 15:163-168.

Behrooz Alizadeh Z, Bobby Koeleman PC (2008). Genetic polymorphisms in susceptibility to Type 1 Diabetes. Clin. Chim. Acta 387:9-17.

Belkhadir J, El Alaoui Z (1993). Approche épidémiologique du diabète en milieu marocain. Médecine du Maghreb 37:35-37.

Brick C, Bennani N, Atouf O, Essakalli M (2006). HLA-A, -B, -DR and -DQ allele and haplotype frequencies in the Moroccan population: a general population study. Transfus. Clin. Biol. 13:346-352.

Bouhours-Nouet N, Coutant R (2005). Diagnosis and characteristics of childhood type 1 diabetes. EMC-Pédiatrie 2:220-242.

Caillat-Zucman S (2001). HLA genes and susceptibility to type 1 diabetes. Pathol. Biol. 49:423-425.

Canosi A, Piancatelli D, Aureli A, Oumhani K, Ozzella G, Del Beato T (2010). Correlation between genetic HLA class I and class II polymorphisms and anthropological aspects in the Chaouya population from Morocco (Arabic speaking). Tissue Antigenes 76:177-193.

Chen BH, Chiang CH, Lin SR, Chao MG, Tsai ST (1999). The influence of age at onset and gender on the HLA-DQA1, DQB1 association in Chinese children with Insulin Dependent Diabetes Mellitus. Hum. Immunol. 60:1131-1137.

Danze PM, Penet S, Fajardy I (1997). Genetic approach for insulin-dependent diabetes mellitus in clinical practice. Ann. Biol. Clin. 55:537-544.

Ikegami H, Kawabata Y, Noso S, Fujisawa T, Ogihara T (2007). Genetics of type 1 diabetes in Asian and Caucasian populations. Diabetes Res. Clin. Pr. 77:116-121.

Izaabel H, Garchon H-J, Beaurain G, Biga M, Akhayat O, Bach J-F et a (1996). Distribution of HLA class II alleles and haplotypes in insulin-dependent Moroccan diabetics. Hum. Immunol. 49:137.

Kanga U, Vaidyanathan B, Jaini R, Menon PSN, Mehra NK (2004). HLA haplotypes associated with Type 1 Diabetes Mellitus in north Indian children. Hum. Immunol. 65:47-53.

Krokowski M, Bodalski J, Bratek A, Boitard C, Caillat-Zucman S (1998). HLA Class II-associated predisposition to Insulin-Dependent Diabetes Mellitus in a Polish population. Hum. Immunol. 59:451-455.

Millot G (2008). Understand and realize the statistical test using a “R”. Manual for beginners. De Boeck Publishing.

Nepom GT, Erlich H (1991). MHC class-II molecules and autoimmunity. Annu. Rev. Immunol. 9:493-526.

Oumhani K, Canossi A, Piancatelli D (2002). Sequence-based analysis of HLA-DRB1 polymorphism in METALSA Berber and Chaouya Arabic- speaking groups from Morocco. Hum. Immunol. 63:129-138.

Park YS, Wang CY, Ko KW, Yang SW, Park M, Yang MCK (1998). Combinations of HLA DR and DQ molecules determine the susceptibility to Insulin-Dependent Diabetes Mellitus in Koreans. Hum. Immunol. 59:794-801.

Parry CS, Brooks RB (2008). A new model defines the minimal set of polymorphism in HLA-DQ and –DR that determines susceptibility and resistance to autoimmune diabetes. Biol. Direct. 3:42.

Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G (2009). and the EURODIAB Study Group Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. Lancet 373:2027-2033.

Platz P, Jakobsen BK, Morling N, Ryder LP, Svejgaard A, Thomsen M, Christy M, Kromann H, Benn J, Nerup J, Green A, Hauge M (1981). HLA and DR antigens in genetic analysis of insulin-dependent diabetes mellitus. Diabetologia 21:108-115.

Sheely MJ (1992). HLA and insulin-dependent diabetes. A protective perspective. Diabetes 41:123-129.

Thomson G (2001). An overview of the genetic analysis of complex diseases, with reference to type I diabetes. Best Pract. Res. Cl. En.

15:265-277.
Thomson G, Robinson WP, Kuhner MK, Joe S, Mac Donald MJ, Gottschall JL (1988). Genetic heterogeneity, mode of inheritance, and risk estimate for joint study of caucasians with insulin-dependent diabetes mellitus. Am. J. Hum. Genet. 43:799-816.
Valdheim CM, Zilder A, Ratter JI, Langba L, Shulman IA, Spenner MR (1989). Different HLA haplotypes in Mexicans with IDDM. Diabetes Care 12:477-500.
Witt CS, Eisenberg S, Soskolne WA, Shapira L, Sela MN, Battat S (1994). HLA polymorphism in Moroccan Jewry. Hum. Immunol. 40:61-67.
Zung A, Elizur M, Weintrob N, Bistritzer T, Hanukoglu A, Zadik Z (2004). Type 1 Diabetes in Jewish Ethiopian immigrants in Israel: HLA Class II immunogenetics and contribution of new environment. Hum. Immunol. 65:1463-1468.