Transfusion and Apheresis Science 49 (2013) 200–204

Contents lists available at SciVerse ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Association of HLA-DR-DQ polymorphisms with diabetes in Tunisian patients

Najiba Fekih Mrissa [a,*], Meriem Mrad [a], Haroun Ouertani [b], Makrem Baatour [c], Aycha Sayeh [a], Brahim Nsiri [a], Khaled Lamine [c], Borni Zidi [b], Nasreddine Gritli [a]

[a] Laboratory of Molecular Biology, Department of Hematology, Military Hospital of Tunisia, Tunisia
[b] Department of Endocrinology, Military Hospital of Tunisia, Tunisia
[c] Department of Emergency, Military Hospital of Tunisia, Tunisia

ARTICLE INFO

Article history:
Received 13 May 2012
Received in revised form 1 October 2012
Accepted 10 January 2013

Keywords:
HLA class II haplotypes
Type I diabetes

ABSTRACT

Objective: Type 1 diabetes (T1D) is a polygenic disease whose principal locus is the human leukocytes antigen (HLA) region. The aim of this study was to evaluate HLA DR-DQ alleles and to asses them as risk factors for type 1 diabetes in the Tunisian population.
Materials and methods: A total of 119 subjects with diabetes were tested for HLA class II alleles and compared with 292 healthy controls. HLA DRB1 and DQB1 alleles were genotyped using polymerase chain reaction sequence-specific primers (PCR-SSPs).
Results: The results revealed that the most susceptible haplotypes are the DRB1*03-DQB1*02 ($p_c < 10^{-3}$) and DRB1*0401-DQB1*0302 ($p_c = 0.001$). (p_c denotes Bonferroni corrected probability values.) The most protective haplotypes are DRB1*11-DQB1*03, DRB1*07-DQB1*02, and DRB1*13-DQB1*06 ($p_c = 0.0026$, $p_c = 0.0065$, and $p_c = 0.02$ respectively). Our results showed some particularities unique to Tunisians, there was a lack of a significant protective effect of the DRB1*15-DQB1*06 haplotype that usually is the dominant combination associated with protection in most other populations.
Conclusion: Tunisian diabetic patients share the most susceptible and protective HLA haplotypes with Caucasians and those in neighbor Mediterranean countries. This is most likely explained by the history and admixture events of Tunisia and North Africa.

1. Introduction

The association of specific alleles at the DRB1 and DQB1 loci with type 1 diabetes (T1D) has been well established in many different populations. Worldwide studies revealed that the frequencies of the different DR-DQ haplotypes and their associations with, or protection against, diseases varied with the ethnic and geographic origins of the populations [1,2].

Disease predisposition involves multiple genes with the most relevant susceptibility occurring in the region of HLA-DR and -DQ genes. They contribute approximately 40–50% of the heritable risk for T1D [3].

The precise mechanisms by which HLA alleles lead to predisposition to, or protection from, type I diabetes are not fully understood but might be related to the differential binding affinity of autoantigenic peptides to distinct polymorphic alleles [4,5].

Todd et al. proposed that HLA-DQB1 was the main diabetogenic gene in the HLA complex [6]. However, the allelic forms of HLA-DRB1 were found to influence the risk of DQB1 susceptibility alleles [7,8].

Currently, the genetic risk of insulin-dependent diabetes mellitus (IDDM) can best be defined by the presence of particular DRB1 and DQB1 alleles. In white Caucasians, heterozygosity for haplotypes encoding DRB1*03-DQB1*02 and DRB1*04-DQB1*0302 are associated with the highest

* Corresponding author. Address: Laboratory of Molecular Biology, Department of Hematology, Military Hospital of Tunisia, 1008 Mont Fleury Tunis, Tunisia. Tel.: +216 22510488; fax: +216 70762084.
E-mail address: fnajiba@yahoo.fr (N. Fekih Mrissa).

1473-0502/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.transci.2013.01.012

genetic risk. In contrast, nearly dominant protection is conferred by the DRB1*15-DQB1*06 haplotype [9,10]. If T1D is assumed to be determined by similar genetic mechanisms in all ethnic groups, recurrent associations of markers with disease should be seen across all groups, despite variations in linkage disequilibrium.

Tunisia, a North African country, has an intermediate incidence rate of type 1 diabetes (5–9.99/100,000 per year). This is in contrast with the highest incidence rate found, for example, in northern Europeans and the lowest incidence found in populations in China and South America [11]. Tunisians are a good example of a highly genetically-diversified population due to great racial admixture [12]. In this regard, Tunisians present an opportunity to study the susceptibility to type I diabetes that has not been previously studied in a large group of such patients.

The aim of the present study was to investigate the association of HLA-DRB1 and DQB1 haplotypes with adult T1D to explore further the contribution of the HLA class II polymorphism to the disease incidence in Tunisia.

2. Patients and method

2.1. Subjects

The study population comprised 119 unrelated subjects (82 male and 37 female) diagnosed to have type 1 diabetes according to the diagnostic criteria established by the National Diabetes Data Group.

Their mean age at onset was 22.92 ± 9.77 years and the sex ratio (males/females) was 2.21. All patients were recruited from the Endocrinology Department of the Military Hospital. A total of 292 unrelated, non-diabetic, healthy subjects (197 male and 95 female) were recruited as the control group. The controls had a mean age of 36.95 ± 8.08 years at sampling and the sex ratio (males/females) was 2.07.

The controls had a negative family history for type 1 diabetes and autoimmune diseases (based on questionnaire responses) and had normal postprandial glycemia.

Patients and controls were native to various regions of Tunisia. The study protocol was approved by the ethics committee of the Military Hospital of Tunisia and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from each participant.

2.2. HLA typing

Subjects with type 1 diabetes and controls were typed for HLA-DRB1 and HLA-DQB1 alleles. Peripheral blood was obtained from patients and healthy subjects.

Genomic DNA was isolated using the QIAamp®DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany) using standard laboratory protocols. (One Lambda Inc., Canoga Park, CA, USA). HLA class II genotyping was performed using low-resolution DRB1 and DQB1 sequence-specific primer (SSP) amplification in all individuals following the manufacturer's protocols [13].

After PCR amplification, the total PCR reaction volume was loaded directly onto a 2.5% agarose gel stained with ethidium bromide and viewed under ultraviolet light. One Lambda DNA/LMT software version 3.98 was used to detect specific DRB1 and DQB1 alleles. The extended haplotypes were assigned on the basis of known linkage disequilibrium patterns in Tunisia and other Caucasian populations [14,15].

2.3. Statistical analysis

HLA allele frequencies were determined by gene counting. Haplotype frequencies were estimated using the EM algorithm and deviations from Hardy–Weinberg equilibrium were both performed using the Arlequin v.3.1 software (http://cmpg.unibe.ch/software/arlequin3). All data was analyzed using the Statistical Package for the Social Sciences v.16 (SPSS Inc., Chicago, IL). The chi-square test or, when required, Fisher's exact test was used for comparison of the frequencies between groups. Bonferroni's correction for multiple tests was performed. The threshold of significance for probability values p_c was 0.05.

3. Results

The disease was observed more often in men than woman with a ratio of 2.21. This ratio is greater than normally expected (1.14) due to the preponderance of males in the military circle seeking treatment at the Military Hospital. The mean age of disease onset was 22.92 years. A comparison of the frequencies of relevant HLA alleles in patients and controls is given in Table 1. The allelic frequencies were in Hardy–Weinberg equilibrium in all the samples and P values were adjusted by applying the Bonferroni correction (p_c). At the DRB1 locus, HLA-DRB1*03 was increased significantly in patients compared to controls (36.55% vs. 15.58%; $p_c < 10^{-3}$), followed by DRB1*04

Table 1
Allele frequency in diabetic patients and controls. (a) HLA-DRB1 and (b) HLA-DQB1.

HLA	% D patients ($2n = 238$)	% Controls ($2n = 584$)	p_c
(a) HLA-DRB1 type			
DRB1*01	3.36	6.84	0.09
DRB1*03 (s)	36.55	15.58	**$<10^{-3}$**
DRB1*04 (s)	22.68	11.98	**$<10^{-3}$**
DRB1*07 (p)	7.98	16.6	**0.01**
DRB1*11 (p)	4.20	13.69	**$<10^{-3}$**
DRB1*13 (p)	8.40	15.92	**0.04**
DRB1*14	5.46	2.39	0.47
DRB1*15	3.36	7.19	0.4
(b) HLA-DQB1 type			
DQB1*02 (s)	37.81	25.17	**$<10^{-3}$**
DQB1*03	26.47	29.96	0.31
DQB1*04	11.34	10.61	0.76
DQB1*05 (p)	10.08	18.32	**0.03**
DQB1*06 (p)	5.46	15.92	**$<10^{-3}$**

D, Diabetic; p_c, Bonferroni corrected probability value; significant p_c is in bold, $p_c < 0.05$; (s), confers susceptibility; (p), confers protection.