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# Family-based association study of HLA class II with type 1 diabetes in Moroccans

## *Étude d'association familiale entre les gènes HLA classe II et le diabète type 1 dans la population marocaine*

A. Drissi Bourhanbour [a,b,*], N. Benseffaj [a,b], S. Ouadghiri [a,b], R. Razine [c], A. Touzani [d], A. Belafraj [d], M. Essakalli [a,b]

[a] *UPR d'immunologie, faculté de médecine et de pharmacie, Rabat Institut, avenue Mohamed Belarbi El Alaoui, BP 6203, 10000 Rabat, Morocco*
[b] *Unité d'immunologie, service de transfusion sanguine et d'hémovigilance, hôpital d'enfants-Rabat, Rabat Institut, CHU Ibn-Sina, rue Lamfadel Cherkaoui, BP 6527, Rabat, Morocco*
[c] *Laboratoire de biostatistique, recherche clinique et epidémiologie, faculté de médecine et de pharmacie, Rabat Institut, avenue Mohamed Belarbi El Alaoui, BP 6203, 10000 Rabat, Morocco*
[d] *Service de diabétologie pédiatrique, hôpital d'enfants-Rabat, Rabat Institut, CHU Ibn-Sina, rue Lamfadel Cherkaoui, BP 6527, Rabat, Morocco*

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ABSTRACT

*Background.* – The T1D is a multifactorial disease; with a strong genetic control. The human leukocyte antigen (HLA) system plays a crucial role in the autoimmune process leading to childhood diabetes. About 440,000 of the childhood population of the world (1.8 billion children under 14 years of age), have type 1 diabetes, and each year an additional 70,000 develop this disorder. The objective of this study was to investigate the distribution of HLA class II in Moroccan families of diabetic children to identify susceptibility alleles of the Moroccan population.
*Subjects and methods.* – We included in this study, Moroccan families who have at least one child with T1D. The age of onset of diabetes was less than 15 years. HLA class II (DRB1* and DQB1*) was carried out by molecular biology techniques (PCR-SSP and PCR-SSO). The FBAT test (family-based association test) was used to highlight the association between T1D and the HLA-DRB1* and -DQB1* polymorphism.
*Results.* – The association of HLA class II (DRB1*, DQB1*) in type 1 diabetes was analyzed in fifty-one Moroccan families, including 90 diabetics. The results revealed that the most susceptible haplotypes are the DRB1*03:01–DQB1*02:01, DRB1*04:05–DQB1*03:02 ($Z = 3.674$, $P = 0.000239$; $Z = 2.828$, $P = 0.004678$, respectively). And the most protective haplotype is the DRB1*15–DQB1*06.
*Conclusion.* – This is the first family-based association study searching for an association between HLA class II and T1D in a Moroccan population. Despite the different ethnic groups forming Morocco, Moroccan diabetics share the most susceptible and protective HLA haplotypes with other Caucasians populations, specifically the European and Mediterranean populations.


*Mots clés :*
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RÉSUMÉ

*Introduction.* – Le diabète de type 1 (DT1) est une maladie multifactorielle avec un important contrôle génétique. Le système HLA joue un rôle primordial dans le processus auto-immun conduisant au DT1. Dans le monde, environ 440 000 enfants (1,8 milliards d'enfants âgés de moins de 14 ans) ont le DT1 et chaque année 70 000 enfants supplémentaires développent ce désordre. L'objectif de ce travail était d'étudier la distribution des molécules HLA de classe II (DRB1*, DQB1*) chez des enfants diabétiques pour identifier les allèles de susceptibilité et de protection vis-à-vis du DT1 chez la population marocaine.

* Corresponding author. UPR d'immunologie, faculté de médecine et de pharmacie, Rabat Institut, avenue Mohamed Belarbi El Alaoui, BP 6203, 10000 Rabat, Morocco.
*E-mail address:* drissiasmaa@yahoo.fr (A. Drissi Bourhanbour).

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*Matériel et méthodes.* – On a inclus dans cette étude des familles marocaines qui ont au moins un enfant atteint DT1. L'âge d'appariation du diabète était inférieur à 15 ans. Le typage HLA classe II (DRB1* et DQB1*) a été réalisé par des techniques de biologie moléculaire (PCR-SSP et PCR-SSO). Le test FBAT (Family-Based Association Test) a été utilisé pour mettre en évidence l'association entre DT1 et le polymorphisme HLA-DRB1* et HLA-DQB1*.

*Résultats.* – L'association HLA classe II (DRB1*, DQB1*) et le diabète type 1 a été étudiée chez 51 familles marocaines, incluant 90 diabétiques. L'analyse des donnés par FBAT montre que les deux haplotypes associés au DT1 dans la population marocaine sont le DRB1*03:01–DQB1*02:01, DRB1*04:05–DQB1*03:02 ($Z = 3{,}674$, $P = 0$, 000239; $Z = 2{,}828$, $P = 0$, 004678, respectivement). L'haplotype le plus protecteur est DRB1*15–DQB1*06.

*Conclusion.* – Même si la population marocaine est formée de plusieurs groupes ethniques, elle partage les mêmes haplotypes HLA de susceptibilité et de protection vis-à-vis du DT1 avec d'autres populations caucasiennes et plus spécifiquement les populations européenne et méditerranéenne.



## 1. Introduction

Type 1 diabetes (T1D) is a polygenic autoimmune disease in which the insulin-secreting pancreatic beta cells are selectively destroyed by T cells [1]. Type 1 diabetes is the third most prevalent chronic disease of childhood. About 440,000 of the childhood population of the world (1.8 billion children under 14 years of age), have type 1 diabetes, and each year an additional 70,000 develop this disorder [2]. According to the estimates by the Moroccan Ministry of Health, 100,000 Moroccans have type 1 diabetes, including 10,000 children [3]. The autoimmunity of T1D is provided by the presence of antibodies against islet cells (ICA), insulin (IAA), glutamic acid decarboxylase (GADA), and tyrosine-phosphatase (IA2-Ab). Genome-wide association studies have identified over 40 polymorphisms that are associated with T1D [4]. However, the major susceptibility for T1D has been mapped to the HLA class II genes HLA-DRB1* and -DQB1* [5,6]. Both susceptible and protective DRB1*–DQB1*haplotypes exist in all populations [7] but differences between populations have been shown. A strong association of HLA-DRB1*03–DQB1*02:01 and of -DRB1*04:01/04:02/04:05–DQB1*03:02 haplotypes with T1D have been established among Caucasians [8–10], and DRB1*09:01–DQB1*03:03 haplotype in Japanese patients [11]. The majority of these studies are case-control. In our work, we have used a family-based association design. Compared to the case-control study designs, family-based association designs are attractive, since they test for both linkage and association. The family-based association designs can also avoid the potential confounding effects of population stratification by using the parents as controls for the case (the affected offspring) and are convenient in refining linkage findings in family samples [12]. Up to now, the association of T1D with HLA locus was not investigated in Moroccan families of diabetic children.

The objective of this study was to investigate the distribution of HLA class II DRB1* and DQB1* alleles in Moroccans families of diabetics children, to identify susceptibility alleles and to refine genetic knowledge of the Moroccan population. The aim is to be able to provide early and adequate care for the subjects at risk.

## 2. Subjects and methods

### 2.1. Subjects

We included in this study fifty-one Moroccan families with at least one child with type 1 diabetes. The families were recruited between 2009 and 2011 from the department of endocrinology of the Children's Hospital of Rabat. They consisted of 38 multiplex-sibs families (at least two affected children) and 13 simplex families (only one diabetic child).

They contain a total of 229 individuals among which 90 are diabetics. The median age was 17 (range: 2–41). The age of onset of T1D was less than 15 years. All subjects gave consent to participate in this study which was approved by the Ethics Committee for Biomedical.

### 2.2. HLA typing

HLA typing was performed on samples derived from both parents, affected children, and unaffected children in all families. Genomic DNA was first extracted and purified from peripheral blood samples collected in 5% EDTA using a commercial kit (Qiagen). Low resolution HLA class II (DRB1* and DQB1*) typing was performed by polymerase chain reaction-sequence specific primers (PCR-SSP) using micro generic HLA DNA typing trays (One Lambda) according to the manufacturer's protocol for all families. In a second step, the DRB1* high-resolution typing was done by polymerase chain reaction sequence-specific oligonucleotide (PCR-SSO) for 24 families. Whereas, the DQB1* was genotyped by using PCR-SSP. All analyzes were carried out in the laboratory of immunohistocompatibility of the Ibn-Sina University Hospital of Rabat, Morocco.

### 2.3. Statistical analysis

The family-based association test (FBAT) software was used to evidence association between HLA-DRB1* and -DQB1* polymorphisms and T1D. This method is an extension of the original transmission disequilibrium test (TDT) [13], a genetic analysis conducted on family trios (affected child and both parents), which assesses whether one allele is inherited in affected children at a rate greater than that predicted. The FBAT allows inclusion of data that cannot be analyzed in the strict TDT, such as families that are missing a parent. Advantages of these methodologies over other population-based approaches include that they test for both association and linkage, and are not susceptible to population stratification, which can introduce confounding factors in case-control studies [14].

The FBAT statistic was calculated under an additive model and for bi-allelic (each allele against all others) mode of testing. Positive $Z$-statistic of single locus and haplotype FBAT indicated that a specific single locus and haplotype was more frequently transmitted to patients with DT1 in informative families than expected under the null hypothesis of no linkage and no association. The negative sign of $Z$-statistic indicates that the frequency of transmitted genotype is negative association with susceptibility to T1D. Haplotype FBAT software was also used to estimate haplotype frequencies. The probability ($P$) value was considered significant at a $P$-value under 0.05.

## 3. Results

### 3.1. HLA-DRB1* allele association in Moroccan patients with DT1

Table 1 shows the HLA-DRB1* alleles frequencies in Moroccan families of diabetic children. A total of eight HLA-DRB1* alleles have been identified. The most frequent HLA-DRB1* alleles were HLA-DRB1*04 (32.5%) and -DRB1*03 (27.7%). Indeed, the FBAT analyses showed that the DRB1*04 and DRB1*03 alleles were significantly associated with DT1 ($Z = 3.942$, $P = 0.000081$; $Z = 3.069$, $P = 0.002146$, respectively). At the opposite, DRB1*11 and DRB1*15 were negatively associated with susceptibility to T1D ($Z = -3.207$, $P = 0.001341$; $Z = -2.751$, $P = 0.005944$, respectively).

### 3.2. HLA-DQB1* allele association in Moroccan patients with DT1

The distribution of alleles frequencies of HLA-DQB1* in Moroccan families of diabetic children are shown in Table 2. We