Original article

HLA-DRB1/DQB1 susceptibility for autoimmune polyglandular syndrome type II and III in south of Tunisia

Susceptibilité HLA-DRB1/DQB1 aux polyendocrinopathies auto-immunes type II et III dans le Sud tunisien

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Résumé

Objectifs. -- L’objectif de notre étude était de déterminer les marqueurs HLA DR-DQ de susceptibilité et de protection dans une population tunisienne de malades atteints de polyendocrinopathies auto-immunes (PEA).

Patients et méthodes. -- Soixante-deux malades non apparentés atteints de PEAII (n = 20) et PEAIII (n = 42) et 146 témoins sains non apparentés du sud tunisien ont été génotypés pour les allèles HLA-DRB1* et HLA-DQB1* du gène HLA class II grâce à une technique de polymerase chain reaction-sequence specific primers (PCR-SSP). Résultats. -- L’allèle HLA-DQB1*03:02 (p = 0,02 ; OR = 2,98) était relativement associé avec les PEAII alors que les allèles HLA-DRB1*03 (p = 3,10^{-6} ; OR = 4,28) et HLA-DQB1*02:01 (p = 0,04 ; OR = 1,95) étaient significativement associés avec les PEAIII. L’étude haplotypique HLA-DRB1*;DQB1* a montré une fréquence élevée des haplotypes DRB1*04;DQB1*03:02 et DRB1*03;DQB1*02:01 chez les patients atteints de PEAII (p = 4,10^{-3} ; OR = 3,31 et p = 0,03 ; OR = 2,74, respectivement), tandis que les patients atteints de PEAIII était significativement associés uniquement avec l’haplotype DRB1*03;DQB1*02:01 (p = 7,2 10^{-8} ; OR = 4,71).

Conclusion. -- Nos données suggèrent que la variation des allèles et des haplotypes HLA de classe II pourrait être un facteur génétique impliqué dans la susceptibilité aux polyendocrinopathies auto-immunes.

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Mots clés : Maladie d’Addison ; Polyendocrinopathies auto-immunes ; Antigènes des leucocytes humains ; Diabète de type 1 ; Thyroïdite auto-immune

Abstract

Objectives. -- The aim of our study was to investigate the association of HLA-DRB1 and HLA-DQB1 alleles with autoimmune polyglandular syndromes (APS) type II and III in a southern Tunisian population. Patients and methods. -- Sixty-two unrelated patients with APSII (n = 20) and APSIII (n = 42) and 146 healthy controls were genotyped for HLA class II alleles (DRB1\*, DQB1\*) by PCR-SSP technique. Results. -- An increased frequencies of HLA-DQB1\*03:02 (P = 0,02 ; OR = 2,98) in APSII patients, HLA-DRB1\*03 (P = 3,10^{-6} ; OR = 4,28) and HLA-DQB1\*02:01 (P = 0,04 ; OR = 1,95) in APSIII patients were found compared to healthy controls. Study of the HLA-DRB1\*;DQB1\* haplotype frequencies showed a higher occurrence of DRB1\*04;DQB1\*03:02 and DRB1\*03;DQB1\*02:01 in APSII patients (P = 4,10^{-3} ; OR = 3,31 and P = 0,03 ; OR = 2,74 respectively) whereas APSIII was only associated with DRB1\*03;DQB1\*02:01 (P = 7,2 10^{-8} ; OR = 4,71).

Conclusion. -- Our data suggest that the variation in class II HLA alleles and haplotypes could be a genetic factor involved in the susceptibility of APS syndrome.

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Keywords: Addison’s disease; Autoimmune polyglandular syndrome; Human leukocyte antigen; Type 1 diabetes; Autoimmune thyroiditis

1. Introduction

Autoimmune polyglandular syndromes (APS) are rare immune endocrinopathies characterized by the coexistence of at least two endocrine gland insufficiencies that are based on
autoimmune mechanisms [1]. Three major subtypes of APS are distinguished according to age of onset, characteristic patterns of disease combinations and different modes of inheritance [2]. APSI is characterized by the occurrence of at least two of the following three components: Addison’s disease, hypoparathyroidism, and candidiasis [2]. APSII is defined as a combination of Addison’s disease, autoimmune thyroid disease and/or type 1 diabetes whereas APSIII summarizes a combination of autoimmune diseases other than Addison’s disease and hypoparathyroidism [2].

In contrast to APSI, which is a monogenic disease [3–6]; several genes may be involved in the etiology of APSII and III in particular HLA genes. In fact, HLA association with APS has been studied in different populations [7–9]. However, no data have been yet reported on the susceptibility of these genes in North African APSII and III patients. Therefore, the aim of the present study was to investigate the susceptibility impact of HLA class II DRB1*, DQB1* alleles and DRB1*:DQB1* haplotypes in Tunisian patients with APSII and APSIII.

### 2. Patients and methods

#### 2.1. Subjects

Blood samples for DNA extraction and genotyping were collected from 62 unrelated patients with APSII \( n = 20 \) and APSIII \( n = 42 \) recruited from the department of endocrinology in the Hedi Chaker Hospital, University of Sfax, Tunisia, with a mean age at the onset of the disease of 32 ± 10.98 (range: 18–62) for APSII and 33 ± 14.2 (range: 7–64) for APSIII.

All the subjects were natives, born and live in the south of Tunisia. Female/male ratios were 16/4 for APSII and 28/14 for APSIII. The clinical diagnosis and patient characteristics are summarized in Tables 1–3.

The diagnosis of the different endocrinopathies was mainly based on clinical criteria as well as the presence of the different specific antibodies for each disease. All patients with Grave’s disease had anti-TSH receptor antibodies (TRAb) (Euroimmun®, Germany). All patients with Hashimoto
thyroïtis had anti-thyroglobuline (TG) and/or thyroperoxidase (TPO) antibodies (Binding Site®, UK). All diabetic patients were positives for at least one β cell autoantibody: islet cell antibody (ICA) (Euroimmun®, Germany) and/or glutamic acid decarboxylase antibody (GADA) (Euroimmun®, Germany) and/or IA2 antibody (IA2A) (Euroimmun®, Germany). All patients with premature ovarian failure had anti-ovarian antibodies (BIO-RAD®, France–USA).

Addison’s disease: after exclusion of other etiologies, autoimmune Addison’s disease was diagnosed by adrenocortical insufficiency and the detection of anti-adrenocortical antibodies (ACA) (BIO-RAD®, France–USA).

One hundred forty-six unrelated healthy controls were randomly collected from the south of Tunisia with a mean age of 42 years. There was no individual or family history of autoimmune disease.

The study protocol has been approved by the local ethics committee (the ethics committee of the Habib Bourguiba, Sfax), and informed consent was obtained from all healthy controls and patients.

2.2. HLA-DRB1* and HLA-DQB1* genotyping

Genomic DNA was obtained from 10 mL of EDTA peripheral blood using a standard phenol-chlorophorm protocol. HLA-DNA typing (DRB1* and DQB1*) was performed by a polymerase chain reaction-sequence specific primer (PCR-SSP) technique using One Lambda® Kits (Kittredge-street, Canoga-Park, USA).

Polymorphic fragments of DRB1* and DQB1* were amplified by the PCR reaction. The obtained fragments were analyzed in a 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet illumination. The reaction results were read and analyzed using the kit instructions.

2.3. Statistical analysis

The frequencies of HLA class II gene polymorphic alleles in patients and controls were compared using the Chi-Square ($\chi^2$) test. P values were considered as statistically significant only after the Bonferroni correction ($P_c$) for the number of assayed alleles of each locus (13 for DRB1* and 7 for DQB1*). Relative risks were calculated as odds ratios (OR) using 2 × 2 contingency tables.

3. Results

3.1. Patient’s characteristics

Among the 20 patients with APSII, there were 16 women and four men. All of them had Addison’s disease associated with other endocrinopathies. Twenty-eight of 42 APSIII patients were women and 14 were men. All of them had type 1 diabetes associated with AITD. After screening the 62 unrelated patients with APS, 16 patients with APSII and 37 patients with APSIII were identified with completely documented case histories. The age of onset of the individual component diseases of APSII and III and the time frame between the onset of the first and the second endocrinopathy were shown in Tables 2 and 3.

3.2. HLA-DRB1* and HLA-DQB1* association in APS type II and III compared to healthy controls

Allele frequencies for HLA-DRB1* and HLA-DQB1* are shown in Table 4. For HLA-DRB1*, 13 different alleles were noted (*01, *03, *04 and *07–*16) while only five different alleles were noted for HLA-DQB1* (*02–*06).

HLA-DRB1* allelic differences were seen between APSII and III patients and healthy controls. In APSII patients, we found a small increase in the frequency of HLA-DRB1*04 and HLA-DQB1*03:02 compared to healthy controls whereas in APSIII patients, HLA-DRB1*03 and HLA-DQB1*02:01 were found to be significantly more frequent compared to controls.

In both groups of patients, HLA-DRB1*13 and HLA-DQB1*06 were decreased compared to the controls. But after Bonferroni’s correction, differences were significant only for HLA-DQB1*03:02 ($P = 2.10^{-2}$; OR = 2.98) in APSII patients, and both HLA-DRB1*03 ($P = 3.10^{-6}$; OR = 4.28) and HLA-DQB1*02:01 ($P = 4.10^{-2}$; OR = 1.95) in APSIII patients.

3.3. DRB1*:DQB1* haplotype associations in APSII and III patients

Examination of the HLA-DRB1*:DQB1* haplotype frequencies showed a significant association of HLA-DRB1*04:DQB1*03:02 and HLA-DRB1*03:DQB1*02:01 with APSII ($P = 4.10^{-3}$; OR = 3.31 and $P = 0.03$, OR = 2.74 respectively) whereas APSIII was associated only with DRB1*03:DQB1*02 ($P = 7.2.10^{-5}$; OR = 4.71) (Table 5).
Allele frequencies for HLA-DRB1* and HLA-DQB1* in APSII and III patients and APS patients stratified by endocrinopathies compared to healthy controls.

## Table 4

<table>
<thead>
<tr>
<th>Allele</th>
<th>PEAII</th>
<th>PEAIII</th>
<th>AITD</th>
<th>DID</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*01</td>
<td>2.5%</td>
<td>2.2%</td>
<td>2.2%</td>
<td>2.2%</td>
<td>2.5%</td>
</tr>
<tr>
<td>DRB1*03</td>
<td>22.5%</td>
<td>22.5%</td>
<td>22.5%</td>
<td>22.5%</td>
<td>22.5%</td>
</tr>
<tr>
<td>DRB1*04</td>
<td>30.0%</td>
<td>30.0%</td>
<td>30.0%</td>
<td>30.0%</td>
<td>30.0%</td>
</tr>
<tr>
<td>DRB1*07</td>
<td>15.0%</td>
<td>15.0%</td>
<td>15.0%</td>
<td>15.0%</td>
<td>15.0%</td>
</tr>
<tr>
<td>DQB1*02</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
</tr>
<tr>
<td>DQB1*03</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
</tr>
<tr>
<td>DQB1*03:02</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
</tr>
<tr>
<td>DQB1*02:01</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
<td>7.5%</td>
</tr>
</tbody>
</table>

P: allele frequencies; +: susceptible allele; c: probability corrected with Bonferroni's correction; OR: odds ratio; AITD: autoimmune thyroid disease; T1D: type 1 diabetes.

Bold and italic figures refer to the susceptibility alleles which were also referred by (+).

3.4. **HLA-DRB1*;DQB1* association in APS patients stratified by endocrinopathies compared to healthy controls**

We found that HLA-DRB1*03 and HLA-DQB1*02:01 were the most frequent alleles in type 1 diabetes (Pc = 10^{-7}; OR = 4.4; Pc = 0.07; OR = 2.55 respectively) and autoimmune thyroiditis (Pc = 5.10^{-6}; OR = 4.33; Pc = 0.02; OR = 2.79 respectively) patients (Table 4).

Our results showed also significant association of the DRB1*04;DQB1*03:02 and DRB1*03:DQB1*02:01 haplotypes with type 1 diabetes (P = 0.036; OR = 1.98; P = 2.10^{-7}; OR = 4.34 respectively) and autoimmune thyroiditis (P = 0.02; OR = 2.02; P = 8.10^{-10}; OR = 4.92 respectively) (Table 5).

3.5. **DRB1*/DRB1* genotypes associations in APS patients**

HLA-DRB1* genotypic differences were seen between patients and controls. In fact, HLA-DRB1*03/DRB1*04 genotype seems to confer susceptibility to all groups of patients. In contrast, HLA-DRB1*03/DRB1*03 genotype is mostly associated with APSIII and its components (type 1 diabetes and autoimmune thyroid disease) (Table 5).

4. **Discussion**

During the last few years, many authors have contributed to investigate clinical, genetic, and immunological aspects of the APS. So far, no study on APSII or III in North African populations has been yet performed.

The demographic and clinical characteristic and disease description of our patients are typical to those shown in the literature [2,9–12].

We performed a case-control study to compare HLA-DRB1*, HLA-DQB1* allele and HLA-DRB1*;DQB1* haplotype polymorphisms in patients with APSII and III versus healthy controls.

Our study did not show significant association of both HLA-DRB1*03, HLA-DRB1*04 alleles with APSII which were, however, reported in other studies. In fact, both HLA-DRB1*03 and HLA-DRB1*04 were found to be increased in American [9,13] and German [14,15] patients with APSII. Whereas, in a British and Finnish studies [16,17], this association was confirmed only for HLA-DRB1*03.

In agreement with several previous studies, we found a significant association of the HLA-DRB1*04;DQB1*03:02 and HLA-DRB1*03:DQB1*02:01 haplotypes in APSII [11,15–18]. Huang et al. [8] performing HLA typing on patients with APSII (Addison’s disease associated with one other disease) showed that the haplotypes DRB1*04:DQB1*03:02 and DRB1*03:DQB1*02:01 were significantly increased in patients compared to healthy controls. But, in the patients lacking β cell autoimmunity (clinical type 1 diabetes and/or ICA or glutamic acid decarboxylase antibodies), only DRB1*03:DQB1*02:01 haplotype was found to be associated suggesting that the association between DRB1*04:DQB1*03:02 and APSII was entirely due to the presence of pancreatic β cell autoimmunity.
In our study, the significant association of DQB1*03:02 allele and DRB1*04;DQB1*03:02 haplotype but not DRB1*04 allele with APSII may suggest that HLA-DQB1*03:02 allele may be the primary allele conferring most of the risk to APSII, whereas HLA-DRB1*04, in linkage disequilibrium with it may have a secondary role.

As far as our APSIII results, we found that HLA-DRB1*03 allele is significantly increased compared to healthy controls which suggests that this allele might be a risk allele for APSIII. This association was confirmed by the fact that the homozygote DRB1*03 provides a higher risk to develop the disease (Table 5).

Those findings are partly consistent with the results obtained from studies performed on some Caucasian populations (Table 6). In fact, exploratory analyses revealed that the HLA-DRB1*03 allele might confer risk for APSIII development in German [19,20] and American populations [21].

HLA-DRB1*04 was not associated with APSIII. The lack of this association contrasts with other studies which reported that HLA-DRB1*04 is a susceptibility allele in APSIII [9,19–21].

Regarding HLA-DQB1* alleles, the HLA-DQB1*02:01 seems to be associated with APSIII. This association was also reported in an American family study [22] which also demonstrates that the risk for APSIII was also conferred by the presence of HLA-DQB1*03:02 allele. The last association was not found in our study.

Our study has shown a significant increase of the DRB1*03; DQB1*02:01 haplotype but not HLA-DRB1*04;DQB1*03:02 in APSIII patients. One study [23] investigating HLA associations in APSIII was performed on 55 multiplexed American families showing preferential transmission of the HLA-DRB1*03;DQB1*02:01 and HLA-DRB1*04;DQB1*03:02 to offspring affected by APSIII.

The HLA locus was also studied in other ethnic groups. In fact, Hashimoto et al. [24] found a significant association of HLA-DRB1*04:05, HLA-DRB1*08:02, and HLA-DQB1*04:01 alleles and HLA-DRB1*04:05-DQB1*04:01 in Chinese populations [25].

Table 5

<table>
<thead>
<tr>
<th>DRB1*/DQB1*</th>
<th>APSII</th>
<th>APSIII</th>
<th>DID</th>
<th>AITD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>OR</td>
<td>P</td>
<td>OR</td>
</tr>
<tr>
<td>DRB1<em>03;DQB1</em>02</td>
<td>3 10⁻²</td>
<td>2.74</td>
<td>7.2 10⁻⁸</td>
<td>4.71</td>
</tr>
<tr>
<td>DRB1<em>04;DQB1</em>03:02</td>
<td>4 10⁻¹</td>
<td>3.31</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DRB1<em>03/DRB1</em>04</td>
<td>3.7 10⁻⁵</td>
<td>11.83</td>
<td>2.6 10⁻⁵</td>
<td>9.68</td>
</tr>
<tr>
<td>DRB1<em>03/DRB1</em>03</td>
<td>NS</td>
<td>NS</td>
<td>10⁻³</td>
<td>7.94</td>
</tr>
</tbody>
</table>

P values were calculated by χ² test. OR: odds ratio.

Table 6

<table>
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<tr>
<th>Studies</th>
<th>HLA susceptibility alleles</th>
<th>HLA susceptibility haplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huang et al. (1996) [8]</td>
<td>APSII</td>
<td>DRB1<em>03, DRB1</em>04</td>
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<td>DRB1<em>03, DRB1</em>04</td>
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<td>Maclaren et al. (1986) [13]</td>
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<td>DRB1*03</td>
</tr>
<tr>
<td>Partanen et al. (1994) [17]</td>
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<td>DRB1*03</td>
</tr>
<tr>
<td>Yu et al. (1999) [18]</td>
<td>APSII</td>
<td>DRB1*04</td>
</tr>
<tr>
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<td>DQB1<em>02, DQB1</em>03:02</td>
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<tr>
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<td>DRB1*08:02</td>
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<td>Katahira et al. (2009) [25]</td>
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<td>DQB1*04:01</td>
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<td>Kim et al. (2003) [26]</td>
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<td>Chuang et al. (1996) [27]</td>
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<td>Our study</td>
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<tr>
<td></td>
<td>DRB1<em>03, DQB1</em>02</td>
<td>DRB1<em>04-DQB1</em>02:01</td>
</tr>
</tbody>
</table>
HLA-DRB1*09:01;DQB1*03:03. HLA-DRB1*04:05;DQB1*04:01 haplotypes in APSIII patients.

Similarly, another Japanese study showed HLA-DRB1*04:05;DQB1*04:01 and HLA-DRB1*09:01;DQB1*03:03 haplotypic association with also a high frequency of HLA-DRB1*08:02;DQB1*03:02 haplotype in APSIII patients compared to healthy controls [25].

In a Taiwanese study, the HLA-DRB1*04:05;DQB1*04:01 haplotype was found to be increased in diabetic patients with autoimmune thyroid disease (APSIII) but not in patients with only type 1 diabetes [26].

The HLA-DQB1*04:01 allele was reported to be associated with patients affected by both type 1 diabetes and autoimmune thyroid disease (APSIII) in two studies from Taiwan [26] and Korea [27].

Taken together, these data suggested that different HLA class II alleles predispose to APSIII in different ethnic groups. However, this predisposition differs between Asian and Caucasian populations. Our results are partly similar to those of Caucasian population but totally different from Asian one. The lack of some associations in our APS patients might be due to their small number or it might reflect genetic differences between populations.

When we stratify our APS patients by endocrinopathies, we observed that both type 1 diabetes and autoimmune thyroid disease patients had significantly higher frequency of DRB1*03;DQB1*02:01 than controls, also, DRB1*04;DQB1*03:02 haplotype was associated with type 1 diabetes, Autoimmune thyroid and Addison’s disease patients. Those results suggest that the high haplotypic frequencies of respectively APSII and III might be due to their components.

5. Conclusion

In conclusion, our data have shown that the HLA alleles and haplotypes may contribute to the genetic susceptibility of APSII and III in the Tunisian population. To confirm these associations, future research should focus on family studies with a larger number of samples. This might offer further knowledge on the inheritance of APSII and III as well as on the familial risk to develop this syndrome in Tunisia.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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