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HLA class II alleles and multiple sclerosis in Tunisian patients

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ABSTRACT

Objective: The aim of our study was to investigate the association of HLA-DRB1 and -DQB1 alleles with multiple sclerosis (MS) in a Tunisian population and their effect on age at onset and disease severity.
Methods: 58 MS patients and 105 healthy controls were genotyped for HLA class II alleles by PCR-SSP technique.
Results: An association of MS with HLA-DRB1*15 was found (14.7% vs 3.8%, OR (95% CI) = 4.34 (1.69–11.39), $p_c = 2.5 \times 10^{-3}$) after Bonferroni's correction. Moreover, the DRB1*15–DQB1*06 (13.8% vs 2.8%, OR (95% CI) = 5.44 (1.92–17.41), $p_c = 1.1 \times 10^{-3}$) and DRB1*04–DQB1*04 (8.6% vs 1.9%, OR (95% CI) = 4.86 (1.36–21.62), $p_c = 0.028$) haplotypes were found to confer a susceptibility to multiple sclerosis.
Conclusion: To our knowledge, this is the first study performed to analyze the association of HLA-DRB1/DQB1 alleles on MS susceptibility in Tunisia. The modern Tunisian gene pool shows some degree of heterogeneity and reflects a significant gene flow from Mediterranean regions.

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) affecting especially young adults mostly in Caucasian populations. Its main features are inflammation, demyelination, gliosis and axonal/neuronal damage [1]. The risk of developing MS is 20–40 times higher for siblings of affected individuals than in the general population [2] and the concordance risk in monozygotic twins is around 30% compared with 3% in dizygotic twins [3]. In most cases, the disease starts with relapses and remissions (85–90%), designed as relapsing–remitting form. Some of them convert after several years of duration in a secondary progressive form which is characterized by a state of slow uninterrupted progression [4]. The cause and pathogenesis of MS are still unclear. Both genetic and environmental factors may play a role in the development of MS. The involvement of immune system by the activation of an inflammatory process leading to macrophage recruitment and subsequent myelin/oligodendrocyte destruction was also described [5,6]. Population and family studies have demonstrated a significant association between human leukocyte antigens (HLA) class II genes and MS. The HLA class II molecules are cell-surface glycoproteins involved in the regulation of immune responses. They are expressed on antigen-presenting cells such as B cells, macrophages and activated T cells. They consist of extracellular and transmembrane regions with a short cytoplasmic tail. The extracellular region is represented by α and β chains of two domains each (α1α2 and β1β2, respectively), and the α1 and β1 domains form the peptide groove. Both polypeptide chains are encoded by genes in the HLA-DP, -DQ, or -DR region of chromosome 6 p21 [7]. The peptide binding to HLA class II molecule implies a core of 9 amino acids and is crucial for the initiation and the regulation of immune responses [8]. The three HLA class II alleles of the DR2 haplotype (DRB1*1501, DRB5*0101 and DQB1*0602) are in strong linkage disequilibrium and were consistently found in most Caucasian populations [9–16]. The exception was found in Sardinian patients with an increased frequency of DRB1*04 and DRB1*03 alleles [17]. To our knowledge, this is the first report studying the association between HLA class II alleles and MS in the Tunisian population. Tunisia is a North-African and a Mediterranean country with an intermediate prevalence of MS [18]. The aim of our study was to investigate the relationship between MS and HLA-DRB1/DQB1 alleles and to evaluate the relation between associated alleles and age at onset and disease severity.

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Table 1
Allelic frequency of HLA-DRB1 subtypes in healthy controls and MS patients.

DRB1 alleles[a]	Patients $2n = 116$ n (frequency)	Healthy controls $2n = 210$ n (frequency)	Odds ratio (95% CI)	p	p_c[b]
DRB1*03	15 (0.129)	41 (0.195)	0.61 (0.31–1.21)	0.13	–
DRB1*04	27 (0.233)	31 (0.148)	1.75 (0.95–3.24)	0.05	–
DRB1*07	14 (0.120)	33 (0.157)	0.74 (0.36–1.51)	0.36	–
DRB1*11	15 (0.129)	33 (0.186)	0.65 (0.32–1.29)	0.18	–
DRB1*13	18 (0.155)	28 (0.133)	1.19 (0.60–2.37)	0.58	–
DRB1*15	**17(0.147)**	**8 (0.038)**	**4.34 (1.69–11.39)**	**0.00042**	**0.00252**
Others	10 (0.086)	30 (0.143)	–	–	

[a] Only DRB1 allele groups with allele frequencies >5% in either cases or controls are analyzed.
[b] Statistically significant associations after Bonferroni's correction are shown in bold.

2. Materials and methods

2.1. Patients and controls

A total of 58 unrelated Tunisian patients with relapsing–remitting MS (ratio female: male 38/20 1.9, the mean age at sampling 33.6 years ± 9.8, range 15–55) were recruited from the neurology and neurosurgery department of Military hospital of Tunis between 2005 and 2008. All patients were diagnosed with clinically definite MS. The mean age at disease onset was 27.6 years (SD 9.3, range 12–51), the mean disease duration was 5.8 years (SD 6.2, range 0–40) and mean Extended Disability Status Scale (EDSS) score was 2.6 (SD 1.48, range 0–6). Diagnostic criteria incorporate magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) and evoked potentials testing. Disability was assessed with Expanded Disability Status scale (EDSS). In our study, the control group is presented by 105 unrelated individuals (ratio female:male 66/36 1.7, the mean age at sampling 35.8 years ± 10.2, range 17–57), recruited from National Center for Blood Transfusion and Military Hospital of Tunis. Patients and healthy controls are natives of various regions of Tunisia.

2.2. HLA typing

Genomic DNA was extracted from peripheral blood samples collected on EDTA anticoagulant using QIAamp®DNA Blood Mini Kit (Qiagen GmbH, Hilden). Low resolution HLA typing was performed by PCR-SSP techniques according to Micro SSP™ DNA Typing Trays DRB/DQB (One Lambda, Inc. Canoga Park, USA). Amplified DNA fragments were migrated on 2.5% ethidium bromide agarose gel and visualised under ultraviolet light. One Lambda DNA/LMT Software version 3.98 was used to detect specific DRB1 and DQB1 alleles.

2.3. Statistical analysis

Alleles frequencies were estimated by the direct counting method. The linkage disequilibrium (LD) determined using Arlequin 3.11 software. Only allele groups and haplotypes with frequencies >5% in either cases or controls were analyzed. The differences between predisposing to and protecting against MS were measured using the odds ratio (OR) method. p values were calculated using Epi Info software version 6.04 with 95% confidence intervals and Fisher's exact correction for small numbers. Corrected probability values (p_c) were determined using Bonferroni's correction by multiplying each p value by the number of allele or haplotype comparisons made: 6 for DRB1 alleles, 5 for DQB1 alleles and 7 for DRB1–DQB1 haplotypes.

3. Results

3.1. Distribution of DRB1 and DQB1 alleles among MS patients

Of the 13 DRB1 allele groups resolved, 6 (DRB1*03, DRB1*04, DRB1*07, DRB1*11, DRB1*13 and DRB1*15) had allele frequencies >5% in either cases or controls. Only these were considered in the analysis. DRB1*04 (23.3%) and HLA-DRB1*15 (14.7%) were the most frequent alleles in MS patients. The HLA-DRB1*15 frequency is significantly higher in MS patients than healthy controls (14.7% vs 3.8%, OR (95% CI) = 4.34 (1.69–11.39), $p_c = 2.5 \times 10^{-3} < 0.05$) even after Bonferroni's correction. HLA-DRB1*04 allele was more frequent in patients (23.3%) than in controls (14.8%); however, the difference was not significant. In healthy control group, HLA-DRB1*03, DRB1*11, DRB1*07 and DRB1*04 subtypes were the most frequent alleles (19.5%, 18.6%, 15.7% and 14.8%, respectively) (see Table 1).

The most frequent DRB1 alleles in controls group were HLA-DRB1*03 (19.5%), DRB1*11 (18%), DRB1*07 (15.7%), DRB1*04 (14.8%) and DRB1*13 (13.3%). In MS patients, the most frequent ones were DRB1*04 (23.3%), DRB1*13 (15.5%), DRB1*15 (14.6%), DRB1*11 (12.9%), DRB1*03 (12.9%) and DRB1*07 (12%). Of the 13 DRB1 allele groups resolved, 6(DRB1*03, DRB1*04, DRB1*07, DRB1*11, DRB1*13 and DRB1*15) had allele frequencies > 5% in either cases or controls. Only these were considered in the analysis. The HLA-DRB1*15 frequency is significantly higher in MS patients than healthy controls (14.7% vs 3.8%, OR (95% CI) = 4.34 (1.69–11.39), $p_c = 2.5 \times 10^{-3} < 0.05$) even after Bonferroni's correction. Among DQB1 alleles, the most frequent allele groups in healthy controls were DQB1*03 (35.7%), DQB1*02 (28%) and DQB1*06 (18%). In MS patients the most frequent ones were DQB1*03 (33.6%), DQB1*06 (25%) and DQB1*02 (24.1%). No association with MS was found in DQB1 allele groups.

3.2. Haplotypes associated with MS

Only haplotypes in linkage desequilibrium were analyzed. The most frequent haplotypes in healthy controls were DRB1*03-DQB1*02 (16.7%), DRB1*11-DQB1*03 (12.4%), DRB1*07-DQB1*02 (11.4%) and DRB1*13-DQB1*06 (8.1%). In MS patients, the most frequent ones were DRB1*04-DQB1*03 (14.7%), DRB1*15-DQB1*06 (13.8%), DRB1*03-DQB1*02 (12.9%), DRB1*07-DQB1*02 (11.2%), DRB1*11-DQB1*03 (10.3%) and DRB1*13-DQB1*06 (8.6%). Haplotypes with frequencies >5% in either patients or controls were compared between both groups. A strong association of multiple sclerosis with DRB1*15-DQB1*06 (13.8% vs 2.8%, OR (95% CI) = 5.44 (1.92–17.41), $p_c = 1.1 \times 10^{-3} < 0.05$) and DRB1*04–DQB1*04 (8.6% vs 1.9%, OR (95% CI) = 4.86 (1.36–21.62), $p_c = 0.028 < 0.05$) haplotypes was found after applying Bonferroni's correction (see Table 2).