Clinical Neurology and Neurosurgery 121 (2014) 19–22

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

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

Association of HLA-DR/DQ polymorphisms with Guillain–Barré syndrome in Tunisian patients

Najiba Fekih-Mrissa[a,b,*], Meriem Mrad[a,c], Anis Riahi[d,e], Aicha Sayeh[a,c], Jamel Zaouali[d,e], Nasreddine Gritli[a,f], Ridha Mrissa[d,e]

[a] Hôpital Militaire Principal d'Instruction de Tunis, Service d'Hématologie, Laboratoire de Biologie Moléculaire, 1008 Montfleury, Tunis, Tunisie
[b] Académie Militaire Fondouk Jédid, 8012 Nabeul, Tunisie
[c] Université de Tunis El Manar, Faculté des Sciences de Tunis, 2092, El Manar, Tunisie
[d] Hôpital Militaire Principal d'Instruction de Tunis, Service de Neurologie, 1008 Montfleury, Tunis, Tunisie
[e] Université de Tunis El Manar, Faculté de Médecine de Tunis, 1007 Tunis, Tunisie
[f] Université de Monastir, Faculté de Pharmacie, 5000 Monastir, Tunisie

ARTICLE INFO

Article history:
Received 16 June 2013
Received in revised form 20 January 2014
Accepted 10 March 2014
Available online 19 March 2014

Keywords:
Neuroimmunology
HLA
Guillain–Barré syndrome (GBS)
Neuropathy

ABSTRACT

Human leukocyte antigen (HLA) alleles have been implicated in many autoimmune diseases. The aim of this study is to assess whether HLA-DR/DQ alleles confer susceptibility to Guillain–Barré syndrome (GBS) in a Tunisian population.

Methods: The HLA-DR/DQ genotyping was performed using polymerase chain reaction sequence-specific primers (PCR-SSP) in 38 patients with GBS and 100 healthy Tunisian control subjects.

Results: GBS in Tunisian patients was found to be associated with the following alleles with these relative patient versus control frequencies (p_c denotes Bonferroni corrected probability values): DRB1*13 (23.68% vs. 9.0%; $p_c = 0.01$), followed by DRB1*14 (22.36% vs.5.5%; $p_c < 10^{-3}$). Two haplotypes, DRB1*14/DQB1*05 and DRB1*13/DQB1*03, were found to be associated with susceptibility to GBS. However DRB1*07/DQB1*02 and DRB1*03/DQB1*02 haplotypes were more frequently observed in controls than in patients (11.5% vs.7.9%; $p_c = 0.007$ and 23% vs. 5.26%; $p_c < 10^{-3}$ respectively). These haplotypes seem to confer protection against the disease.

Conclusion: Our data demonstrated a new GBS predisposition associated with HLA-DRB1*14 and DRB1*13. Theses alleles could be predisposing genetic factors for GBS in the Tunisian population.

1. Introduction

Guillain–Barré syndrome (GBS) is an acute demyelinating disease of the peripheral nerves characterized by progressive weakness that usually follows a viral illness or immunization [1,2].

The exact pathogenetic mechanisms remain uncertain. However, several features suggest an autoimmune component in the etiology of this disease. Both humoral and cell-mediated immune responses have been suspected of playing a role in disease susceptibility [3]. The hypothesis of an autoimmune/immune-mediated cause is supported by nerve biopsy studies that confirmed the presence of lymphocytes and macrophages within the cellular inflammatory peripheral nerve infiltrate [4–6]. The human leukocyte antigen (HLA) complex is crucial for immunity and its polymorphisms are specifically associated with autoimmune inflammatory diseases [7].

Several genetic studies show that the major histocompatibility complex (MHC) genes, especially HLA antigens represent the strongest risk for developing several diseases with autoimmune features [8,9]. Because GBS was thought to have an immune-mediated, possibly autoimmune component, the HLA molecules carried by patients with this disorder have been evaluated in several studies. HLA and GBD association studies have given a variety of associations with conflicting results [10–14]

The aim of the present study was to study the distribution of HLA class II alleles in GBS Tunisian patients.

2. Materials and methods

2.1. Subjects

The study population comprised 38 unrelated patients with GBS (30 male, 8 female) with a mean age ± SD of 39.44 ± 21.65 years. All patients fulfilled standard diagnostic criteria [15]. The criteria

* Corresponding author at: Laboratoire de Biologie Moléculaire, Service d'Hématologie, Hôpital Militaire Principal d'Instruction de Tunis, 1008 Montfleury, Tunis, Tunisie. Tel.: +216 22510488; fax: +216 70762084.
E-mail address: fnajiba@yahoo.fr (N. Fekih-Mrissa).

http://dx.doi.org/10.1016/j.clineuro.2014.03.014
0303-8467/

Table 1
Characteristics of GBS patients.

Characteristics	Number of patients ($n = 38$)	Percentage (%)
Sex		
Female	8	21.05
Male	30	78.99
Age at initial evaluation mean (range)	39.44 ± 21.65 (14–81)	–

required evidence of progressive motor weakness of more than one limb, areflexia, and specific progression (symptoms and signs of motor weakness that develop rapidly but cease to progress by four weeks into the illness), and the exclusion of other causes for neuropathy. Electrophysiological studies and cerebral spinal fluid (CSF) analysis were investigated in all patients to support the diagnosis of GBS. All patients were treated with intravenous immunoglobulin (IVIg) with a dosage of 0.4 g/kg body weight/day for five consecutive days. All but one patient had clear signs of demyelinating neuropathy. One patient died in the acute stage of the disease while in the intensive care department. All other patients recovered fully.

The controls consisted of 100 healthy matched individuals with diverse Tunisian origin similar to the patients, none of whom had any history of GBS, family antecedent with GBS, nor any peripheral neuropathy. All participants gave informed consent before participation in the study.

2.2. HLA-typing by DNA amplification

Genomic DNA was extracted from peripheral blood samples of patients and healthy individuals using the QIAamp®DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany).

Low-resolution HLA typing was performed by PCR-SSP techniques according to Micro SSP DNA Typing Trays DRB/DQB (One Lambda Inc. Canoga Park, CA, USA). Amplified DNA fragments were detected by agarose gel electrophoresis (2.5% agarose gel), stained with ethidium bromide, and UV transillumination. One Lambda DNA/LMT software version 3.98 was used to detect specific DRB1 and DQB1 alleles.

2.3. Statistical analysis

Allele frequencies were estimated by using the direct counting method. 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 analyses related to the case-control study were performed using the Statistical Package for the Social Sciences v.16 (IBM, Armonk, NY, USA). Differences between cases and controls were evaluated by using the chi-square test or Fisher's test for qualitative variables. The odds ratios (OR) and 95% confidence intervals (CI) were also calculated. The Bonferroni method was used to adjust for type I errors due to multiple comparisons. Corrected probability values (p_c) were calculated for the number of comparisons made, i.e., the number of alleles tested. Probability values $p_c < 0.05$ were considered statistically significant (Table 1).

3. Results

The disease was observed more often in men than woman with a ratio of 3.75. The mean age of disease onset was 39.44 years. A comparison of the frequencies of tested HLA alleles in study subjects is given in Table 2. The allelic frequencies were in Hardy–Weinberg equilibrium in all the samples. Probability values (*P* values) were adjusted by applying the Bonferroni correction (p_c). Among DRB1 alleles, HLA-DRB1*13 was increased significantly in patients compared to controls (23.68% vs. 9.0%; $p_c = 0.01$), followed by DRB1*14 (22.36% vs.5.5%; $p_c < 10^{-3}$). These two alleles were associated with an increased risk for GBS. HLA-DRB1*03, however, revealed a marked decrease in frequency (5.26% vs. 21.5%; $p_c = 0.001$) in patients compared to controls, followed by DRB1*07 (10.52% vs. 15.5%; $p_c < 10^{-3}$). These alleles seem to confer protection against GBS. None of the DQB1 alleles tested in this study constituted a statistically significant risk for GBS.

Table 2
Allele frequency in GBS patients and controls (a) HLA-DRB1 and (b) HLA-DQB1.

HLA	% GBS patients ($n = 38$)	% Controls ($n = 100$)	*P*-value
HLA-DRB1 type			
DRB1*01	5.26	7.5	.51
DRB1*03	5.26	21.5	**.001**[a]
DRB1*04	5.26	12.5	.07
DRB1*07	10.52	15.5	**<10(**$^{-3}$[a]
DRB1*11	13.15	13.5	.94
DRB1*13	23.68	9.0	**.002**[a]
DRB1*14	22.36	5.5	**<10(**$^{-3}$[a]
DRB1*15	10.52	12	.73
(b) HLA-DQB1 type			
DQB1*02	21.0	31.0	.1
DQB1*03	44.7	30.0	.1[a]
DQB1*05	7.8	16.0	.08
DQB1*06	26.3	15.5	.19[a]

GBS, Guillain–Barré syndrome; *P*-value, probability value; significant *P*-value is in bold, $P < 0.05$.
[a] Corrected *P*-value.

Only haplotypes exhibiting significant linkage disequilibrium (LD) parameters between alleles were considered for study. Haplotypes with frequencies greater than 5% in either patients or controls were compared between both groups in Table 3. A pronounced frequency of the DRB1*14/DQB1*05 haplotype among patients was observed which indicates a strong predisposing effect toward disease (13.5% vs. 1.5%; $p_c < 10^{-3}$). This haplotype was followed in significance of disease susceptibility by DRB1*13/DQB1*03 (14.75% vs. 4.0%; $p_c = 0.02$).

Among the protective DR-DQ haplotypes, DRB1*07/DQB1*02 and DRB1*03/DQB1*02 were significantly more prevalent among controls in comparison with GBS patients (11.5% vs. 7.9%; $p_c = 0.007$ and 23% vs. 5.26%; $p_c < 10^{-3}$ respectively). These haplotypes seem to confer protection against GBS.

4. Discussion

In this study, we report an association of two haplotypes, DRB1*14/DQB1*05 and DRB1*13/DQB1*03, that have not

Table 3
The frequencies of HLA DR/DQ haplotypes in GBS patients.

HLA DR/DQ allele DRB1*/DQB1	% GBS patients ($n = 38$)	% Controls ($n = 100$)	*P*-value
DRB1*01/DQB1*05	5.26	7.5	.97
DRB1*03/DQB1*02	5.26	23	**.007**[a]
DRB1*04/DQB1*03	5.26	12.0	.09
DRB1*07/DQB1*02	7.9	11.5	**<10(**$^{-3}$[a]
DRB1*11/DQB1*03	10.52	13.0	.57
DRB1*13/DQB1*03	14.75	4.0	**.02**
DRB1*14/DQB1*05	13.5	1.5	**<10(**$^{-3}$[a]
DRB1*15/DQB1*06	10.52	12.5	.82

GBS, Guillain–Barré syndrome; *P*-value, probability value; significant *P*-value is in bold, $P < 0.05$.
[a] Corrected *P*-value.