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Laboratory Study

Association between HLA-DRB1 and myasthenia gravis in a northern Han Chinese population

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ABSTRACT

The cause of myasthenia gravis (MG) is unknown, but it is widely believed to be an autoimmune disease occurring in genetically susceptible individuals. The human leukocyte antigen (HLA) region is considered to be the most important genetic region for MG susceptibility genes. To investigate the association between HLA-DRB1 and myasthenia gravis (MG) in a northern Han Chinese population, a polymerase chain reaction with sequence-specific oligonucleotide probe hybridization method was used to determine the HLA-DRB1 genotypes of 91 patients with MG and 171 healthy individuals. We found that the HLA-DRB1*09 allele was significantly more prevalent among patients with MG than among healthy controls, especially those who experienced early onset of the disease (⩽40 years), those who were seronegative for acetylcholine receptor antibody, and those with ocular MG. The prevalence of the HLA-DRB1*08 allele was significantly lower among patients with MG than among controls. These results indicate that HLA-DRB1*09 might be positively associated and DRB1*08 negatively associated with MG in the northern Han Chinese population.

1. Introduction

Myasthenia gravis (MG) is an autoimmune disorder caused by the activity of antibodies against muscle acetylcholine receptors (AChRs) at the postsynaptic membranes of neuromuscular junctions.[1] MG is characterized by a postsynaptic blockade of nervous transmission, resulting in weakness and easily fatigued striated muscles. MG can be life-threatening when bulbar or respiratory muscles are involved.

Although the specific cause of MG is unknown, the disorder appears to occur in genetically susceptible individuals after exposure to certain environmental factors that have yet to be identified. Progress has been made in characterizing the genetic factors underlying susceptibility to MG. The first study concerning the role of the human leukocyte antigen (HLA) system in MG susceptibility was carried out in 1972, and even today the HLA is frequently referred to as the most important genetic region for MG susceptibility genes.[2]

Associations between several different HLA class alleles and MG have been identified in diverse ethnic groups. At the DRB1 locus, the most frequently reported association is the HLA-DRB1*03 allele found in most European populations.[3,4] HLA-DRB1*09 has also been found to be associated with MG in Venezuelan and Japanese populations.[5,6] Several studies have investigated the association of the HLA allele with demographic variables, but no consistent patterns have emerged so far.

The primary aim of the present study was to investigate the relationship between HLA-DRB1 and MG in a northern Han Chinese population. Associations between HLA-DRB1 and the clinical and demographic characteristics of patients with MG were also evaluated.

2. Subjects and methods

2.1. Patients and controls

A total of 91 unrelated Chinese patients were included in the study. They were enrolled in our hospitals and fulfilled the clinical and electromyographic diagnostic criteria for acquired MG. The clinical and biological features of these cases of MG are listed in Table 1. A total of 171 unrelated healthy individuals with an ethnic

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Table 1
Clinical features of 91 Han Chinese patients with myasthenia gravis

Feature	No. patients (women/men)
Total no.	91 (59/32)
Age at disease onset	
⩽40 years (early onset)	52 (38/14)
>40 years (late onset)	39 (21/18)
Anti-AChR antibody status	74 (49/25)†
Seronegative (<0.4 nM)	30 (19/11)
Seropositive (⩾0.4 nM)	44 (30/14)
Outcome of thymic CT	79 (52/27)†
Thymoma	14 (10/4)
Non-thymoma	65 (42/23)
MG clinical type	
Ocular	34 (19/15)
Generalized	57 (40/17)

AChR = acetylcholine receptor, MG = myasthenia gravis.
† Data were not available for all 91 patients.

background similar to that of the patients with MG were enrolled as controls. Each subject was informed about the study and gave written informed consent for genetic analysis.

2.2. Samples

Peripheral blood samples (5 mL) from controls and patients with MG in either the acute stage or the recurrent stage were collected in ethylenediamine tetraacetic acid. The autoantibody titer was measured by the Medical Diagnostic Institute Laboratory (Berlin, Germany). Genomic DNA was obtained from proteinase K-treated peripheral blood leukocytes using a salting-out procedure.

2.3. HLA-DRB1 genotyping

The second exon of the DRB1 gene was amplified using polymerase chain reaction (PCR) according to a standard protocol. PCR products were separated electrophoretically using a 1.5% agarose gel containing ethidium bromide. Low-resolution genotyping was performed using the CapitalBio HLA Genotyping Services System (CapitalBio, Beijing, China) with 79 sequence-specific oligonucleotide probes based on alleles designated by the World Health Organization Nomenclature Committee for DRB1.[7] Quality control was conducted by the National Institute of Geriatrics.

2.4. Statistical analysis

Mean values were compared using a *t*-test. That HLA-DRB1 allele frequencies did not deviate from Hardy–Weinberg equilibrium was verified using R software. According to the codominant inheritance pattern, differences between groups in the prevalence of each allele were assessed using Fisher's exact test (2-tailed). Odds ratios (OR) with 95% confidence intervals (CI) were calculated, and the level of significance was set at $p < 0.05$. MG is a heterogeneous disease, and different subtypes may have different genetic backgrounds. To test the hypothesis that different HLA-DRB1 alleles are associated with different MG subtypes, we extracted subsets of patients with MG according to clinical and laboratory findings, and the prevalence of HLA-DRB1 alleles in these MG subsets was compared. Subsets were extracted according to age of disease onset (early onset, ⩽40 years *versus* [*vs.*] late onset, > 40 years), clinical type (Osserman classification; ocular MG *vs.* generalized MG), and AChR antibody status (seropositive *vs.* seronegative).

SPSS for Windows version 12.0 (SPSS, Chicago, IL, USA) was used for statistical analysis.

3. Results

3.1. HLA-DRB1 alleles in patients with MG compared to healthy controls

The prevalences of HLA-DRB1 alleles among patients with MG and controls are presented in Table 2. The HLA-DRB1*09 allele was the most frequent allele found in patients with MG, but the HLA-DRB1*15 allele was the most frequent allele found in controls. HLA-DRB1*09 was significantly more prevalent in patients with MG than in controls (36.26% in patients with MG *vs.* 23.98% in controls, OR = 1.80, 95% CI = 1.04–3.14, $p = 0.043$). The prevalence of HLA-DRB1*08 was significantly lower in patients with MG than in controls (6.6% *vs.* 15.2%, OR = 0.39, 95% CI = 0.16–0.99; $p = 0.048$).

3.2. Comparisons according to patient subgroup

When patients with MG were grouped into early onset ($n = 52$) and late-onset patients ($n = 39$), DRB1*09 was found to be significantly more prevalent among early-onset patients (OR = 2.15, $p = 0.033$) than among late-onset patients (Table 3). Conversely, DRB1*08 and DRB1*14 were significantly less prevalent among early-onset patients (OR = 0.11 and 0.29, respectively; $p = 0.007$ and 0.043, respectively). DRB1*07 was significantly more prevalent among late-onset patients with MG (OR = 2.43, $p = 0.030$).

When patients were grouped according to disease type (ocular MG, $n = 34$; generalized MG, $n = 57$), HLA-DRB1*09 was found to be significantly more prevalent in the ocular MG group, (OR = 5.12, $p = 5.35 \times 10^{-5}$) and HLA-DRB1*12 was significantly

Table 2
HLA-DRB1 allele distribution among Han Chinese patients with myasthenia gravis and healthy control subjects

HLA-DRB1 allele	Patients with MG ($n = 91$)		Controls ($n = 171$)		OR (95% CI)	p
	n	%	n	%		
*01	5	5.49	9	5.26	1.05 (0.34–3.22)	1
*03	8	8.79	13	7.60	1.17 (0.47–2.94)	0.812
*04	19	20.88	38	22.22	0.92 (0.50–1.72)	0.876
*07	24	26.37	32	18.71	1.56 (0.85–2.85)	0.157
*08	6	6.59	26	15.20	0.39 (0.16–0.99)	0.048*
*09	33	36.26	41	23.98	1.80 (1.04–3.14)	0.043*
*10	2	2.20	3	1.75	1.26 (0.21–7.67)	1
*11	7	7.69	19	11.11	0.67 (0.27–1.65)	0.516
*12	19	20.88	40	23.39	0.86 (0.47–1.60)	0.756
*13	13	14.29	19	11.11	1.33 (0.63–2.84)	0.553
*14	9	9.89	30	17.54	0.52 (0.23–1.14)	0.105
*15	30	32.97	46	26.90	1.34 (0.77–2.32)	0.319
*16	3	3.30	4	2.34	1.42 (0.31–6.50)	0.697

CI = confidence interval, MG = myasthenia gravis, OR = odds ratio.
* $p < 0.05$ (Fisher's exact test).