Myasthenia gravis with antibodies to muscle-specific kinase : clinical characteristics, epidemiology, and immunological aspects

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IV

Strong association of MuSK-antibody positive myasthenia gravis and HLA-DR14-DQ5

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Abstract

The authors studied the HLA profile of 23 white Dutch patients with muscle-specific kinase antibody-positive myasthenia gravis (MuSK MG) and found an association with HLA-DR14-DQ5 (odds ratio 8.5; 95% CI 3.9-18.7; $p = 4.9 \times 10^{-5}$). Fifty-two percent of the patients carried the DR14 allele compared to 5% percent of the controls ($p = 1.0 \times 10^{-8}$). This association between MuSK MG and a relatively rare HLA haplotype differs from the previously described association of early-onset AChR MG with HLA-B8-DR3.

Introduction

In approximately 90% of patients with generalised weakness, myasthenia gravis (MG) is caused by autoantibodies directed against the nicotinic acetylcholinereceptor (AChR). In 30% to 40% of the patients with generalised MG without anti-AChR antibodies (AChR Ab- MG), antibodies against muscle-specific kinase (MuSK) can be found. MG with anti-MuSK autoantibodies (MuSK MG) comprises a clinical phenotype with marked involvement of oculobulbar, neck and diaphragmatic muscles. [56] Anti-MuSK antibodies are mainly of the immunoglobulin (Ig) G4 subclass, instead of IgG1 and IgG3 in AChR MG. [93]
The presence of other autoimmune diseases in patients with AChR MG and their first-degree relatives suggests a role for a genetic predisposition of which the HLA genotype is the most well known. Early-onset AChR MG without thymoma is strongly associated with the HLA-A1-B8-DR3 haplotype in white Europeans. [20-22] DR16 and DR9 are also linked to AChR MG in white French people. In this cohort, an association with polymorphisms in the gene encoding the α-subunit of the AChR as well as a decreased frequency of the DR7 allele has been found. DR2 has been associated with late-onset AChR MG. We studied HLA polymorphisms in white Dutch patients with MuSK MG.

Methods

Between July 2003 and December 2004, patients known to have acquired generalised AChR Ab- MG from all eight University Medical Centres and five larger general hospitals in the Netherlands were included in a nationwide study of seronegative MG. Patients were selected using computerised diagnosis registrations and asked for informed consent by their attending neurologist. A single investigator (E.H.N.) re-examined all patients to assess present clinical symptoms. Course of the disease and evolution of clinical symptoms in time were evaluated retrospectively by taking a detailed history from each patient, the use of questionnaires and review of patients' charts. Weakness during the onset of clinical symptoms and maximum disease severity was graded according to the Myasthenia Gravis Foundation of America (MGFA) clinical classification. The presence of other autoimmune diseases in the patients and their first-degree relatives was evaluated during the interview. Serum from all patients was obtained and retested for the presence of anti-AChR, anti-MuSK and anti-voltage-gated calcium channel (VGCC) antibodies in Leiden using commercial assays (RSR Ltd., UK) and in Oxford. DNA was isolated from peripheral white blood cells. HLA Class I and II typing was performed with the use of PCR-amplified fragments and biotin-labelled oligonucleotides as previously described. [119] DNA typed DRB1* and DQB1* alleles were translated throughout to their serologic equivalents according to the World Health Organization nomenclature

committee. Frequencies of HLA alleles were compared to those of a previously published group of 2,440 healthy white Dutch blood donors. [120] Distribution of HLA haplotypes was compared to that of 321 individuals from the same control group available for haplotype assignment. For statistical analysis the two-sided Fisher exact test was used. *p* values were corrected for multiple informative comparisons conform the Bonferroni method. Odds ratios (ORs) with 95% CIs were calculated according to the Woolf Haldane test. All patients gave written informed consent before their participation. The Medical Ethical Committees of all hospitals involved approved the study.

Results

Patient characteristics

Seventy-three patients with acquired generalised AChR Ab- MG were found in the Netherlands. Anti-MuSK antibodies were present in 26 of whom 23 white patients (18 women and 5 men) were included. None of these patients had anti-AChR antibodies in at least two assays (range 2 to 14) performed in different laboratories. No anti-VGCC antibodies were found. The mean age at onset was 36.4 years (range 6.0 to 74.5 years). The mean duration of follow up was 10.4 years (range 1.2 to 33.9). The mean time from onset of symptoms until the first period of maximum disease severity was 1.8 years (range 0.25 to 6.75), apart from one patient whose weakness was greatest 32 years after onset. Weakness at onset of symptoms was restricted to the extraocular muscles in 8 (MGFA Class I), predominantly bulbar in 10 (MGFA Class IIb or IIIb), mainly affecting the extremities in 2 (MGFA Class IIa) and mixed generalised in 3 (MGFA Class II). At maximum disease severity, however, bulbar and respiratory muscles were prominently involved in 15 patients (MGFA Class IIb, IIIb and IVb), with a further 7 requiring ventilatory support (MGFA Class V), leaving only 1 patient with severe mixed generalised weakness (MGFA Class IV).

HLA association

The absolute and relative distribution of HLA polymorphisms in patients and controls were calculated (Table 4.1). A highly significant association was found with alleles DR14 (52% *vs*. 5% in healthy controls) and DQ5 (78% *vs*. 35%). Nineteen of 23 patients carried either DR14, or DQ5, or both. The HLA-DR14-DQ5 haplotype was significantly increased among MuSK MG patients (p_c = 4.9 x 10^{-5}, Table 4.2). The frequency of the DQ6 allele was decreased in MuSK MG (22% *vs*. 50%). There was no significant association with the B8-DR3 ancestral haplotype or with HLA Class I alleles.

Table 4.1 Frequencies of HLA alleles in 23 patients with MuSK Ab+ MG.

HLA*	MuSK n *(%)*	Controls n *(%)*	OR	95% CI	*p*	p_c §
A1	4 (17.4)	747 (30.6)	0.5225	0.1868 - 1.4616	0.2539	0.9778
B8	3 (13.0)	554 (22.7)	0.5809	0.1862 - 1.8117	0.3275	0.9974
DR3	2 (8.7)	599 (25.0)	0.3484	0.0937 - 1.2952	0.0885	0.6712
DR1	9 (39.1)	473 (19.7)	2.6601	1.1666 - 6.0657	0.0320	0.3235
DR14	12 (52.2)	127 (5.4)	18.8448	8.2956 - 42.8093	8.5×10^{-10}	1.0×10^{-8}
DR16	2 (8.7)	43 (1.8)	6.1173	1.5960 - 23.4471	0.0701	0.5821
DQ5	18 (78.3)	300 (34.6)	6.3523	2.4271 - 16.6255	3.1×10^{-5}	1.5×10^{-4}
DQ6	5 (21.7)	453 (50.1)	0.2960	0.1132 - 0.7736	0.0098	0.0482

* To allow comparison with a large control population that was typed serologically, DNA typed DR and DQ alleles were translated to their serological equivalents.
§ Corrected *p* value according to the Bonferroni method for multiple comparisons.

Table 4.2 Distribution of HLA-DQ5 associated haplotypes in patients and controls

HLA	MuSK n *(%)*	Controls n *(%)**	OR	95% CI	p_c §
DR14-DQ5	11 (23.9)	23 (3.6)	8.5	3.9 - 18.7	4.9×10^{-5}
DR1-DQ5	9 (19.6)	61 (9.5)	2.4	1.1 - 5.1	n.s.
DR10-DQ5	1 (2.2)	7 (1.1)	2.8	0.5 - 16.5	n.s.
DR16-DQ5	2 (4.3)	7 (1.1)	4.8	1.1 - 20.6	n.s.

* This control cohort consisted of 321 individuals available for haplotype assignment.
§ Corrected *p* value for multiple comparisons.

Related autoimmune diseases

Five patients had another autoimmune disease: two had thyroid disease, one had vitiligo, and two had psoriasis. Four of these five patients carried the DR14-DQ5 haplotype. Eight patients had a first-degree relative with autoimmune disease: three with thyroid disease, one with vitiligo, three with rheumatoid arthritis, and one with psoriasis. The DR14-DQ5 haplotype was found in three of these eight, and three patients had DQ5 in combination with DR1. Only one patient, whose first-degree relative had vitiligo, carried the B8-DR3 ancestral haplotype.

Discussion

We found a highly significant association in MuSK MG with HLA-DR14 and DQ5. This association is different from the known association of the B8-DR3 haplotype with early-onset AChR MG. Our cohort of MG patients is representative of the MuSK MG patients described so far, because clinical signs and symptoms are similar to descriptions by other groups. [56,93]

The HLA-DQ5 allele is in linkage disequilibrium with several HLA-DR alleles including DR1, DR10, DR14, and DR16. Although DQ5 as a group was significantly increased in MuSK MG, no significant association was found for the DR1-DQ5, DR10-DQ5, and DR16-DQ5 haplotypes as shown in Table 4.2. The following DQ5 haplotypes can be observed: DQB1*0501 with DR1 and DR10, DQB1*0502 with DR16 and DQB1*0503 with DR14. These DQ5 molecules differ in their β-chains, which may influence antigen presentation. The association with DR14 is highly significant with an OR of 18.8, and we found one patient with DR14-DQ6, an extremely rare haplotype in white Dutch people, being absent in 321 healthy controls. Therefore, we speculate that the association is explained by the presence of the DR14-DQ5 haplotype rather than by DQ5 as a group.
DR14 is also associated with pemphigus vulgaris in non-Jewish white and Pakistani patients. [121,122] The DR14-DQ5 haplotype is even linked to the presence of pemphigus-specific anti-desmoglein 3 antibodies in relatives of the Pakistani patients. Interestingly, in both pemphigus foliaceus and vulgaris, autoantibodies directed to desmoglein 1 and 3 are mainly of the IgG4 subclass during periods of disease activity. [123] A predominance of the non-complement fixing IgG4 isotype is also found in MuSK MG. [93] In contrast, autoantibodies in AChR MG are mainly IgG1 and IgG3 isotypes and cause complement-induced damage to the postsynaptic membrane. The association of MuSK MG and the HLA DR14-DQ5 haplotype adds to the previously described differences in clinical appearance and IgG subclass distribution of antigen-specific antibodies between MuSK MG and AChR MG, suggesting a different immunopathogenesis for both diseases.

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