Classification of Myasthenia Gravis Based on Autoantibody Status

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Objectives: To investigate the autoantibody status of patients with myasthenia gravis (MG) and to evaluate its usefulness for disease classification.

Design: Retrospective cohort study of patients with MG, who have autoantibodies to receptors and ion channels expressed at neuromuscular junctions and in muscles that impair neuromuscular transmission. One of the autoantibodies studied was a recently identified, novel, MG-specific autoantibody to a voltage-gated potassium (Kv) channel, Kv1.4.

Setting: Keio University Hospital, Tokyo, and Iwate Medical University Hospital, Morioka.

Patients: Two hundred nine patients with MG.

Main Outcome Measures: Anti-Kv1.4 antibody was measured by an immunoprecipitation assay with sulfur 35–labeled extract from rhabdomyosarcoma cells. Antititin antibody was detected with a commercially available enzyme-linked immunosorbent assay.

Results: Anti–acetylcholine receptor, anti-Kv1.4, and antititin antibodies were detected in 150 (72%), 26 (12%), and 50 (24%) of the 209 patients with MG, respectively. All of the patients who were positive for anti-Kv1.4 or antititin antibody were seropositive for the anti–acetylcholine receptor antibody. They were classified into 4 groups based on their status in regard to 3 MG-related autoantibodies: anti-Kv1.4, antititin, and anti–acetylcholine receptor. Clinical associations were found between anti-Kv1.4 and bulbar involvement, myasthenic crisis, thymoma, and concomitant myocarditis and/or myositis; between antititin and older-onset MG; between anti–acetylcholine receptor alone and younger-onset MG; and between seronegativity and ocular MG. In addition, patients with MG in the anti-Kv1.4 group had more severe manifestations of disease than those in the other 3 groups.

Conclusion: Classification of patients with MG based on autoantibody status may be useful in defining clinical subsets.

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AUTOANTIBODIES TO VOLTAGE-gated potassium (Kv) channels are known to be associated with acquired neuromyotonia, Morvan syndrome, and autoimmune nonparaneoplastic limbic encephalitis.[1,2] The serum samples of patients with these diseases mainly target members of Kv1 α subunits: Kv1.1, Kv1.2, or Kv1.6.[1,2] The expression of these subunits that form Kv channels differs in brain and muscle, and a novel myasthenia gravis (MG)–specific autoantibody to a Kv channel, Kv1.4, was recently identified.[3]

The presence of autoantibodies to receptors and ion channels expressed at neuromuscular junctions and in muscle impairs neuromuscular transmission,[4] and autoantibodies to the acetylcholine receptor (AChR) and other targets, including titin, ryanodine receptor, and muscle-specific kinase, have been reported in patients with MG.[4] These MG-related autoantibodies are associated with specific clinical features (ie, antititin with older-onset MG and thymoma,[5-10] anti-Kv1.4 with a severe form of MG and thymoma,[3] and anti–muscle-specific kinase with facial and bulbar muscle involvement in MG).[11] Because MG is heterogeneous in terms of disease expression, including age at onset, thymus pathological features, clinical subsets ranging from an ocular form to a generalized form, and disease severity,[4] identification of these autoantibodies may be useful in classifying disease subsets in patients with MG.

In this study, we measured 3 MG-related autoantibodies, anti-AChR, anti-Kv1.4, and antititin, in Japanese patients with MG and evaluated their usefulness in disease classification.

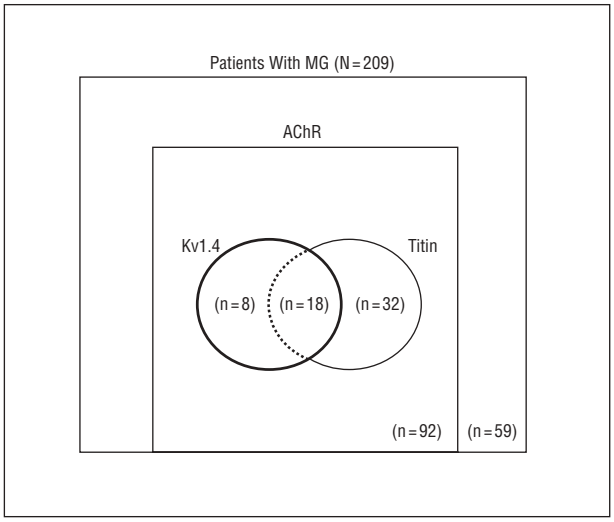

Figure 1. Schema showing the distribution of patients with myasthenia gravis (MG), stratified according to the presence of combinations of anti–acetylcholine receptor (AChR), anti–MG-specific autoantibody to a voltage-gated potassium channel (Kv1.4), and antititin antibodies.

METHODS

PATIENTS

The subjects were 209 Japanese patients with MG (81 men and 128 women) who were being monitored at Keio University Hospital, Tokyo, or Iwate Medical University Hospital, Morioka. The diagnosis of MG was made on the association of the following variables: typical history and signs of fluctuating weakness of voluntary muscles, presence of serum anti-AChR antibody, definite clinical improvement on injection of anticholinesterase, and decremental pattern on repetitive nerve stimulation.[12] The cohort included 61 patients who were evaluated in a previous study.[3] The mean±SD age at antibody determination was 54.0±17.2 years. Extended thymectomy was performed in 107 patients, and histopathologic examination revealed a normal thymus in 27, thymic hyperplasia in 32, and thymoma in 48. The remaining 102 patients also underwent chest computed tomography and/or magnetic resonance imaging, and thymoma was ruled out. The 48 patients with a histologically confirmed diagnosis were diagnosed as having a thymoma. Clinical information on all patients with MG was obtained retrospectively by investigators (S.S. and Y.N.) who were blind to the antibody status of the patients. Serum samples were obtained from 87 patients at diagnosis and from 122 during the subsequent course of their disease. Seventy-two patients received immunosuppressive therapy, and 29 were in remission when the blood sample was collected. The severity of MG at blood sampling was graded according to the system proposed by the Task Force of the Medical Advisory Board of the Myasthenia Gravis Foundation of America,[13] with some modifications: grade 0, no symptoms; grade 1, ocular muscle weakness only; grade 2, mild generalized weakness; grade 3, moderate generalized weakness; grade 4, severe generalized weakness; and grade 5, intubation required. We defined "older-onset MG" as MG onset after the age of 60 years.[14] The myocarditis diagnosis was based on cardiac symptoms without any other cause and typical electrocardiographic findings. The myositis diagnosis was based on clinical symptoms, elevated serum creatine kinase levels, electromyographic findings, and results of a muscle biopsy. The diagnosis was verified by postmortem examination in 1 patient with myocarditis and myositis.

AUTOANTIBODY ASSAYS

Serum anti-AChR antibody was measured by a conventional radioimmunoassay, and values greater than 0.5nM were regarded as positive.[4] Anti-Kv1.4 antibody was determined by an immunoprecipitation assay using sulfur 35–labeled cellular extract as the antigen source, as previously reported.[3] Serum samples that precipitated 70-kDa Kv1.4 from rhabdomyosarcoma cell extracts and not from leukemic cell extracts were regarded as positive. Anti-Kv1.4 antibody was not detected in patients with polymyositis and thymoma without MG and health controls.[3] Antititin antibody was detected with a commercially available enzyme-linked immunosorbent assay in which a recombinant MGT30 protein was used as the antigen (DLD Diagnostika GNBH, Hamburg, Germany).[6,7] The optical densities value calibrated at 450 nm greater than 1.0 was regarded as positive according to the manufacturer's protocol. All blood samples and clinical information were obtained after the patients had given their informed consent, and the study was approved by the institutional review boards of each hospital.

DATA ANALYSIS

Statistical analysis was performed using a statistical software program (StatView 5.0; SAS Institute Inc, Cary, North Carolina). Categorical variables were compared by the χ^2 test. Continuous variables were compared by analysis of variance. Disease severity was compared by the Mann-Whitney test. $P < .05$ was considered significant.

RESULTS

Figure 1 shows the distribution of the patients with MG, stratified by their status in regard to the 3 MG-related autoantibodies. Anti-AChR, anti-Kv1.4, and antititin antibodies were detected in 150 (72%), 26 (12%), and 50 (24%) of the 209 patients with MG, respectively. All of the patients who were positive for anti-Kv1.4 or antititin antibody were seropositive for anti-AChR antibody. The serum of 18 of the 50 antititin-positive patients (36%) also contained anti-Kv1.4 antibody, while the serum of 8 of 159 antititin-negative patients (5%) contained anti-Kv1.4 antibody ($P < .001$). Based on the distribution of the 3 MG-related autoantibodies, the patients with MG were grouped into 5 subsets: an anti-Kv1.4–positive/antititin-positive/anti-AChR–positive subset (n=18), an anti-Kv1.4–positive/antititin-negative/anti-AChR–positive subset (n=8), an anti-Kv1.4–negative/antititin-positive/anti-AChR–positive subset (antititin group; n=32), an anti-Kv1.4–negative/antititin-negative/anti-AChR–positive subset (anti-AChR group; n=92), and an anti-Kv1.4–negative/antititin-negative/anti-AChR–negative subset (seronegative group; n=59). Because there were no statistically significant differences in demographic or clinical features between the anti-Kv1.4–positive/antititin-positive/anti-AChR–positive subset and the anti-Kv1.4–positive/antititin-negative/anti-AChR–positive subset, they were combined into an anti-Kv1.4 group (n=26) in the subsequent analysis.

We then compared the demographic and clinical features of these 4 groups, stratified according to MG-related antibody status (**Table**). There were no differences in sex distribution, but the antititin group was

Table. Clinical Features of Patients With MG Stratified According to Anti-AChR, Anti-Kv1.4, and Antititin Antibody Status[a]

Variable	Anti-Kv1.4 Group (n = 26)	Antititin Group (n = 32)	Anti-AChR Group (n = 92)	Seronegative Group (n = 59)	Overall *P* Value
Male-female ratio	13:13	16:16	31:61	21:38	.22
Age at disease onset, mean±SD, y	49.5±10.3	59.5±16.9	37.5±18.4	43.1±19.0	<.001[b]
Older onset	4 (15)	17 (53)	14 (15)	12 (20)	<.001[c]
Thymoma	19 (73)	14 (44)	15 (16)	0	<.001[d]
Limited to ocular form	4 (15)	6 (19)	21 (23)	30 (51)	<.001[e]
History of bulbar involvement	19 (73)	10 (31)	23 (25)	4 (7)	<.001[f]
History of myasthenic crisis	8 (31)	4 (13)	8 (9)	1 (2)	.001[g]
Concomitant autoimmune diseases					
Autoimmune thyroid diseases[h]	1 (4)	1 (3)	9 (10)	10 (17)	.10
Alopecia areata	2 (8)	1 (3)	2 (2)	0	.20
Myocarditis and/or myositis	4 (15)	0	0	0	<.001[i]
Rheumatoid arthritis	0	0	3 (3)	0	.27
Acquired neuromyotonia	1 (4)	0	0	0	.07

Abbreviations: AChR, acetylcholine receptor; Kv1.4, myasthenia gravis (MG)–specific autoantibody to a voltage-gated potassium channel.

[a] Data are given as number (percentage) of each group unless otherwise indicated. The anti-Kv1.4 group was positive for anti-AChR antibody, positive for anti-Kv1.4 antibody, and positive or negative for antititin antibody; the antititin group, positive for anti-AChR antibody, negative for anti-Kv1.4 antibody, and positive for antititin antibody; the anti-AChR group, positive for anti-AChR antibody and negative for anti-Kv1.4 and antititin antibodies; and the seronegative group, negative for anti-AChR, anti-Kv1.4, and antititin antibodies.

[b] $P=.03$ between the anti-Kv1.4 and antititin groups, $P=.002$ between the anti-Kv1.4 and anti-AChR groups, and $P<.001$ between the antititin and anti-AChR groups and between the antititin and seronegative groups.

[c] $P<.001$ between the anti-Kv1.4 and antititin groups, between the antititin and anti-AChR groups, and between the antititin and seronegative groups.

[d] $P=.02$ between the anti-Kv1.4 and antititin groups; $P<.001$ between the anti-Kv1.4 and anti-AChR groups, between the anti-Kv1.4 and seronegative groups, between the antititin and seronegative groups, and between the anti-AChR and seronegative groups; and $P=.003$ between the antititin and anti-AChR groups.

[e] $P=.003$ between the anti-Kv1.4 and seronegative groups and between the antititin and seronegative groups and $P<.001$ between the anti-AChR and seronegative groups.

[f] $P=.003$ between the anti-Kv1.4 and antititin groups, $P<.001$ between the anti-Kv1.4 and anti-AChR groups and between the anti-Kv1.4 and seronegative groups, and $P=.004$ between the antititin and seronegative groups and between the anti-AChR and seronegative groups.

[g] $P=.007$ between the anti-Kv1.4 and anti-AChR groups and $P<.001$ between the anti-Kv1.4 and seronegative groups.

[h] Graves disease or Hashimoto thyroiditis.

[i] $P=.03$ between the anti-Kv1.4 and antititin groups, $P=.002$ between the anti-Kv1.4 and anti-AChR groups, and $P=.007$ between the anti-Kv1.4 and seronegative groups.

significantly older at onset than any of the other groups, resulting in a higher frequency of older-onset MG in the antititin group than in the other 3 groups. By contrast, the anti-AChR group was significantly younger than the anti-Kv1.4 and antititin groups at disease onset. There were clear differences among the 4 groups in the frequency of thymoma ($P<.001$). By contrast, ocular MG was significantly more common in the seronegative group than in the other 3 groups. Bulbar involvement and myasthenic crisis were most common in the anti-Kv1.4 group and least common in the seronegative group. **Figure 2** shows the distribution of MG severity according to the Task Force of the Medical Advisory Board of the Myasthenia Gravis Foundation of America classification at blood specimen collection, stratified by MG-related autoantibodies. Patients with MG in the anti-Kv1.4 group had more severe manifestations of disease than those in the other 3 groups. No statistically significant differences were detected among the antititin, anti-AChR, and seronegative groups. Myocarditis or myositis was a significantly ($P<.001$) more common concomitant autoimmune disease in the Kv1.4 group than in the other 3 groups.

COMMENT

In the present study, we demonstrated that patients with MG can be subgrouped into distinct clinical subsets based on the presence of combinations of 3 MG-related autoantibodies: (1) an anti-Kv1.4 group with a severe form of MG graded by the Task Force of the Medical Advisory Board of the Myasthenia Gravis Foundation of America classification and a high rate of bulbar involvement and myasthenic crisis, thymoma, and concomitant myocarditis and/or myositis; (2) an antititin group with older-onset MG and thymoma; (3) an anti-AChR group with younger-onset MG; and (4) a seronegative group with ocular MG. This classification may be useful for predicting the disease course of patients with MG in clinical settings and deciding on the treatment regimen. In particular, anti-Kv1.4 antibody may be a useful marker for the MG subset with severe neuromuscular manifestations and concomitant myocarditis and/or myositis that requires more intensive immunosuppressive therapy.

Previous studies[3,5,7,9-11] mainly evaluated clinical associations with 1 particular MG-related autoantibody. The antititin antibody was frequently examined in patients with MG in many studies, and its frequency in patients with MG as a whole was 20% to 40%, and increasing to 60% to 80% in patients with older onset or thymoma.[5-10] We were also able to confirm the associations between antititin antibody and older-onset MG and thymoma. It is widely accepted that titin antibodies are a sensitive marker of thymoma in patients with MG younger than 60 years, and their presence in patients without thymoma identifies a special subgroup with older-onset MG.[15] In addition, some reports[6,10,16] have described an asso-

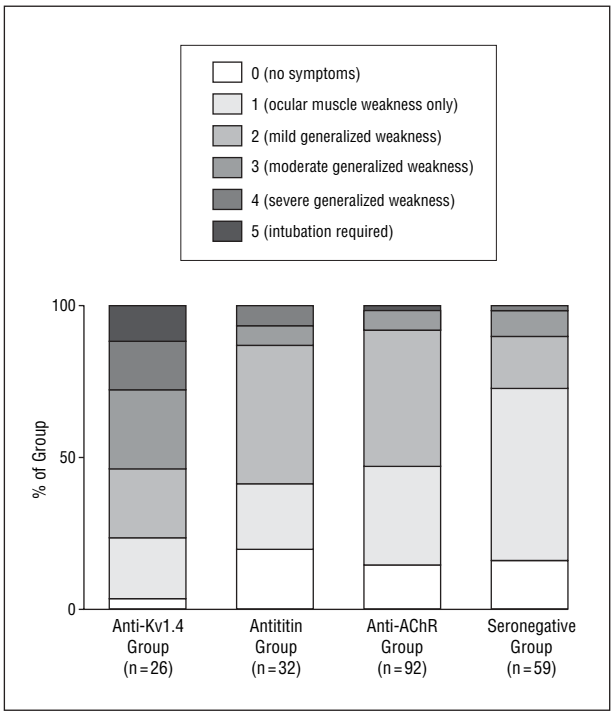

Figure 2. Severity of myasthenia gravis (MG), stratified according to the presence of MG-related autoantibodies. Disease severity was graded from 0 to 5 according to the system proposed by the Task Force of the Medical Advisory Board of the Myasthenia Gravis Foundation of America. Differences between 2 groups were analyzed with the Mann-Whitney test. Patients with MG in the anti-Kv1.4 group had more severe manifestations of disease than did those in the antititin group ($P = .004$), the anti-AChR group ($P < .001$), and the seronegative group ($P < .001$). AChR indicates acetylcholine receptor; and Kv1.4, MG-specific autoantibody to a voltage-gated potassium channel.

ciation between the presence of antititin antibody and severe MG and unsatisfactory outcome after thymectomy. We speculate that this association is explained by the tendency for anti-Kv1.4 and antititin antibodies to both be present in the same individual.

By combining the results of testing for multiple MG-related autoantibodies, it was possible to classify the patients with MG into 5 subgroups. Although we defined the seronegative group as negative for anti-AChR, anti-Kv1.4, and antititin antibodies, other autoantibodies may be detected in these patients. Patients with MG can be classified into more than 5 disease subsets by including combinations with anti–muscle-specific kinase and anti–ryanodine receptor antibodies, which were not included in our study. Anti–ryanodine receptor antibody, in particular, has been reported to be associated with myocarditis and/or myositis.[17]

Although we did not examine the serial change of autoantibody status during the clinical course, immunosuppressive therapy may suppress the appearance of autoantibody. A prospective study is needed to determine whether the MG subgroup classification based on autoantibody status at diagnosis can be used to predict the outcome in patients with MG.

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