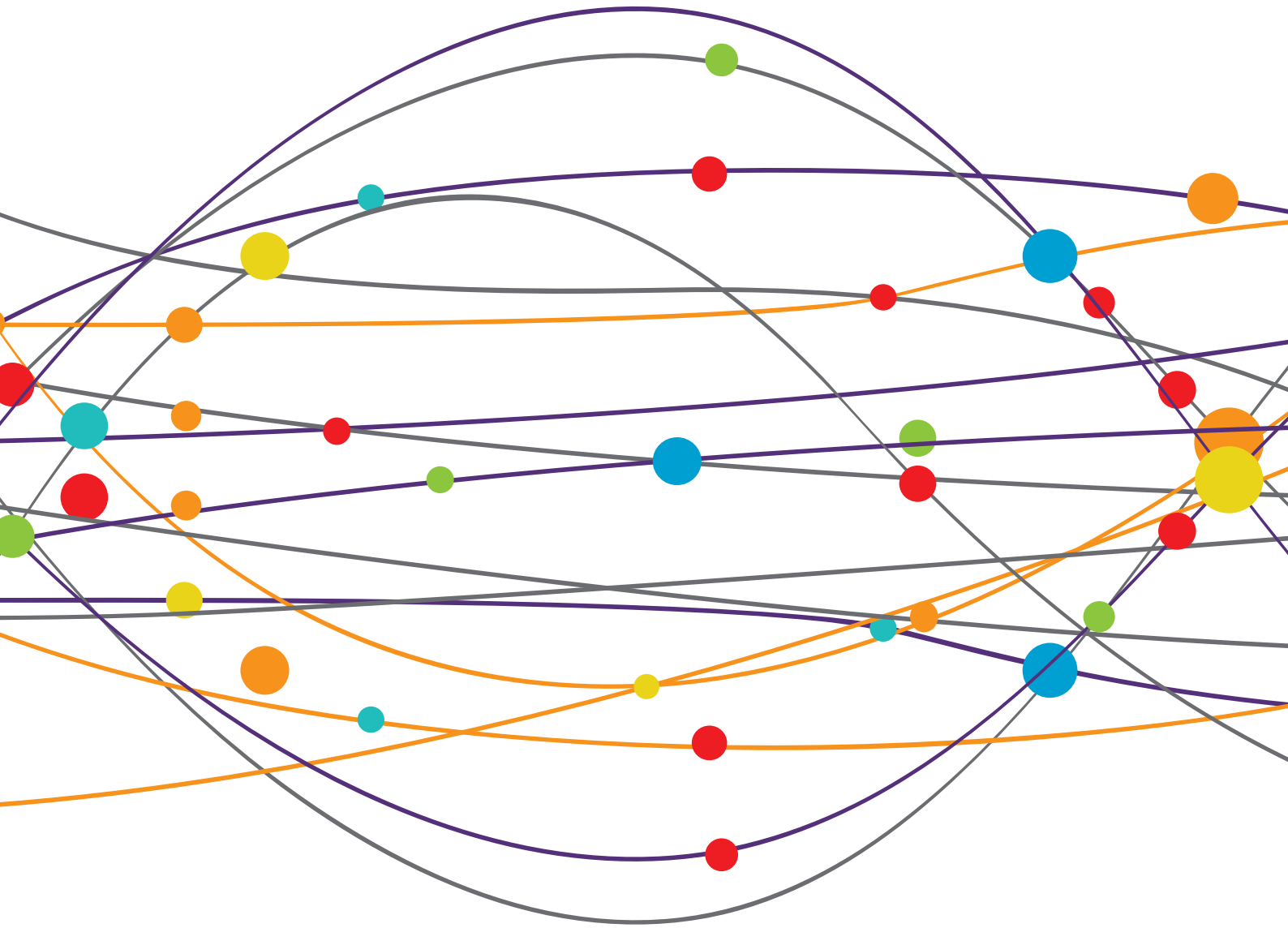
PHENOTYPES OF MYASTHENIA GRAVIS

EDITED BY: Hai-Feng Li, Nils Erik Gilhus, Huan Yang and Xiangjun Chen
PUBLISHED IN: Frontiers in Neurology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-83250-321-8
DOI 10.3389/978-2-83250-321-8

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

PHENOTYPES OF MYASTHENIA GRAVIS

Topic Editors:
Hai-Feng Li, Capital Medical University, China
Nils Erik Gilhus, University of Bergen, Norway
Huan Yang, Central South University, China
Xiangjun Chen, Fudan University, China

Citation: Li, H.-F., Gilhus, N. E., Yang, H., Chen, X., eds. (2022). Phenotypes of Myasthenia Gravis. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-83250-321-8

Table of Contents

frontiers | Frontiers in Neurology

TYPE Editorial
PUBLISHED 14 September 2022
DOI 10.3389/fneur.2022.1025183

OPEN ACCESS

EDITED AND REVIEWED BY
Giovanni Meola,
University of Milan, Italy

*CORRESPONDENCE
Hai-Feng Li
drlhf@163.com

SPECIALTY SECTION
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

RECEIVED 22 August 2022
ACCEPTED 01 September 2022
PUBLISHED 14 September 2022

CITATION
Li H-F, Gilhus NE, Yang H and Chen X (2022) Editorial: Phenotypes of myasthenia gravis.
Front. Neurol. 13:1025183.
doi: 10.3389/fneur.2022.1025183

Editorial: Phenotypes of myasthenia gravis

Hai-Feng Li[1]*, Nils Erik Gilhus[2,3], Huan Yang[4] and Xiangjun Chen[5,6]

[1]Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China, [2]Department of Neurology, Haukeland University Hospital, Bergen, Norway, [3]Department of Clinical Medicine, University of Bergen, Bergen, Norway, [4]Department of Neurology, Xiangya Hospital, Central South University, Changsha, China, [5]Department of Neurology, Huashan Hospital, Fudan University, Shanghai, China, [6]Human Phenome Institute, Fudan University, Shanghai, China

KEYWORDS

myasthenia gravis, phenotype, personalized treatment, mechanism, immunotherapy

Editorial on the Research Topic
Phenotypes of myasthenia gravis

Disease phenotypes are observable and recognizable traits of diseases, which are not limited to hereditary diseases. A single essential feature or a specific combination of features in a disease can be defined using qualitative and quantitative descriptions, with the goal of understanding the full spectrum of disease phenotypes. This will lay the foundation for a reasonable diagnostic process, for assessing illness severity and treatment efficacy, and for identifying the individualized characteristics of patients to guide a precise personalized treatment.

Myasthenia gravis (MG) is a prototypical autoimmune disease with well-defined autoantibodies that target the neuromuscular junction. However, MG exhibits a high degree of phenotypic heterogeneity. Demographic characteristics, extent of muscle involvement, disease progression, presence and level of pathogenic antibodies, immunologic profiles, quantitative measurements of severity, comorbidities, subgroup classification, drug efficacy and long-term stability are all phenotypic characteristics that differ among individual patients. This special topic, including 13 original research articles, two brief research reports, two reviews, and one opinion article, all relevant to the above-mentioned phenotypic characteristics, contributes to an improved understanding and assessment of MG phenotypes.

Phenotypic description

A comprehensive description of phenotypic characteristics provides an integrative understanding of the disease and highlights the clinical features that should be paid attention in clinical practice.

Short-term and long-term prognosis after a first acute dyspnea episode that occurred 12 (4∼34.5) months after disease onset were reported in a study of 86 MG patients. Early-onset MG and precipitating respiratory infection were found as independent

risk factors for progression to myasthenic crisis, which occurred in 41.9% of the included patients. However, with proper immunosuppressive therapy, the patients had an overall good prognosis (Huang et al.). In a study of 796 MG patients naïve to immune therapies, ≥1 concurrent autoimmune diseases were found in 11.6%. Compared to the general population, a significantly higher incidence of various autoimmune diseases was found, especially for hyperthyroidism, immune thrombocytopenic purpura, autoimmune hemolytic anemia, autoimmune hepatitis, and polymyositis. MG patients with concurrent autoimmune diseases were predominantly female, younger at MG onset, and they seldom had MuSK antibodies. Furthermore, they tended to have a mild clinical presentation of MG, including a lower proportion of previous myasthenic crisis and a higher proportion of MGFA Class I at onset (Shi, Huan et al.). Thymoma has a high frequency of concurrent autoimmune diseases, and MG in particular. Previous studies indicate that there is a difference in the concurrent autoimmune disease profile between MG patients with and without thymoma (1, 2). Shi, Huan et al. found that thymoma was less common in MG patients with concurrent autoimmune diseases.

With the increased use of immune-checkpoint inhibitors (ICIs) for cancer treatment, the incidence of neurological immune-related adverse events is growing. ICI-related MG (irMG) is relatively common and has a high fatality rate (3). In a case series combined with a systematic review, 63 irMG patients and 380 idiopathic MG patients were compared. Higher MGFA class and higher QMGS (i.e., more severe disease) were observed in irMG patients compared to idiopathic MG. More irMG patients had concurrent myositis or myocarditis. An unfavorable disease outcome was found in 35% of the irMG patients. Myocarditis, higher MGFA class and QMG score were associated with an unfavorable disease outcome in irMG patients (Shi, Tan et al.).

The International Consensus Guidance for Management of MG calls for the latest evidence relevant to the management of MG to be assessed (4). Some phenotypic subgroups have been little studied. Studies focusing on the natural history and treatment response in patients of very early and very late onset ages, and with ocular onset, are collected in this special topic (Bi et al.; Zhao et al.; Zheng et al.; Zhou et al.). The studies attempt to define indicators of treatment response and prognosis applying real world data and using a retrospective design. Furthermore, phenotypic differences in juveniles with ocular manifestations of MG in different populations were discussed in detail and with a special focus on pathogenic mechanisms and treatment responses in a comprehensive review (Heckmann et al.).

Healthcare resource utilization (HCRU) and costs associated with generalized MG were reported in a study of 41,940 patients of the United States. Mean HCRU and costs were higher for newly diagnosed patients and patients with exacerbation events. For patients who experienced MG crisis, HCRU and costs markedly increased during the 12 months immediately before the crisis event compared with the two preceding years. The costs increased further during the 12 months following the index crisis event (Phillips et al.). This study provided valuable data on health economics in MG patients of generalized phenotype.

Phenotypic biomarkers

Autoantibodies in patients with MG can target all subunits of the AChR at both their extracellular and intracellular regions. In one study, a combination of immunoadsorption with cell-based assays (CBA) was used to examine the specificity of the autoantibodies against the extracellular parts of AChR molecule in AChR antibody positive patients defined by RIPA. Antibodies against intracellular region were found probably not related to neuromuscular transmission impairment, although a detailed analysis was not available. Moreover, the autoantibodies were divided into distinct groups based on their target, highly relevant for disease severity. The antibodies against non-α1 epitopes were found in patients with a milder disease, and they were inversely correlated with MGFA class. A combination of RIPA and CBA is recommended by the authors for the follow-up of MG. The former method is to be used for the quantification of the antibodies and the latter for the identification of fluctuations in culprit antibodies (Michail et al.). This study represents an important advance in the understanding of AChR antibodies in MG. However, the generation mechanism and diagnostic value of anti-intracellular region antibodies remain to be elucidated.

Pathogenic and MG-associated antibodies represent main phenotypic variables in MG subgroup classification with the purpose of individualized or stratified treatment. Whether antibodies combined with clinical variables are useful in deciding optimal therapy was examined in a study of 188 treatment-naïve generalized MG patients who were single AChR antibody positives, dual AChR and LRP4 antibody positives, and dual AChR and titin antibody positives. Patients with AChR plus titin antibodies had more severe MG and progressed faster than those with AChR plus LRP4 antibodies and those with only AChR antibodies. However, all patients responded well to immunotherapy and had relatively good prognosis regardless of the three antibody groups (Chen et al.). MG patients with MuSK antibodies represent a distinct subgroup. Originally regarded as a severe MG, there are now reports of patients with a relatively benign course, or with overlapping phenotypes between MuSK-MG and AChR-MG (5). In a study of 69 MuSK-MG patients, comparison of clinical features and outcomes at 3, 6, and 12 months after onset were conducted among those with different onset age (early-onset, late-onset, and very-late-onset). The very-late-onset subgroup had the highest frequency of limb, bulbar and respiratory involvement, which might prompt earlier usage of potent immunosuppressive therapy. Most MuSK-MG

patients benefited from rituximab treatment regardless of age at onset (Zhou et al.).

Immunologic biomarkers such as LINC00680, a long non-coding RNA, were found associated with the QMG score in a small cohort of MG patients (Liu et al.). More researches on the association between immunological profiles and treatment effects and prognosis of MG are needed.

Phenotypic correlation on treatment response

Glucocorticoid (GC) represents the mainstay of MG treatment. However, prolonged usage of high-dose GC leads to various adverse effects. Therefore, there is consensus that low-dose GC is the aim for long-term maintenance of long-term therapy. Clinical factors related to relapses during GC tapering or after withdrawal were investigated in a study of 125 MG patients who were stable on GC monotherapy. Relapse during the steroid reduction was found to be associated with drug-reducing speed. Furthermore, relapses were more prevalent in patients with onset symptoms of bulbar weakness (Su et al.). In a study of 149 GC-resistant childhood-onset MG patients, 75.8% responded well to tacrolimus. One month after initiating tacrolimus, QMG and ADL scores had improved and the prednisone dose was reduced. QMG and ADL scores continued to improve throughout the study. The prednisone treatment was eventually stopped in 78.8% of the patients. Thymus pathology and pre-intervention status were found to be independent predictors of tacrolimus efficacy (Bi et al.).

Predictors of secondary generalization in patients with vary late onset MG were explored in 69 patients. Absence of immunotherapy was found as the only predictor of secondary generalization in those with pure ocular onset (Zhao et al.). In a study of 53 MG patients with MuSK antibodies, the relapse rate was significantly lower in patients receiving GC combined with other immunosuppressants than in those with only GC. Of all potential associated factors, only the use of additional immunosuppressants was associated with a lower relapse risk (Tan et al.). Among 70 very late onset MG patients, no significant differences in outcomes were observed between those receiving tacrolimus treatment alone and those with tacrolimus combined with GC. Nor did the outcome differ between the tacrolimus group and the group that had never used tacrolimus or used tacrolimus for <3 months. No significant associations were found between tacrolimus administration and clinical outcomes. Although high quality of life was observed in patients treated with tacrolimus, which is better over another in using directly tacrolimus mono-therapy or combining GCs first to stabilize the disease and then taking tacrolimus alone for maintenance therapy is not clear (Zheng et al.).

Treatment resistance to GC is an important phenotypic variable in the treatment of MG. Presently, treatment-resistant patients can only be defined retrospectively. To what degree early treatment response predicts long-term refractoriness is unknown. In an integrative review, definition of GC resistance in MG was discussed in relevance to potential mechanisms, including the underlying MG pathology explaining no response to GC, the susceptibility to GC adverse effects that compromise the ability to achieve therapeutic doses, and the phenotypic and genetic variations that limit the response to GC. Moreover, the authors emphasized that neither patient nor clinician should be content with just an improvement from a poor baseline and with still considerable disability. The aim should be expecting a situation close to minimal manifestation status (MMS) (Kaminski and Denk). Some generalized MG patients are difficult to treat, but true non-responsive and refractory disease hardly occurs. However, extraocular muscles are vulnerable to be impaired in shorter periods due to functional denervation. Hence, definitions for difficult-to-treat or refractory generalized MG do not apply to ocular involvement in MG. Based on the treatment outcomes of extraocular muscles in MG and presumed pathogenic mechanisms, a definition for treatment-resistant ophthalmoplegia was proposed in a comprehensive review (Heckmann et al.).

Methods to assess MG phenotypes

Measurements of disease status and criteria of treatment response represent important phenotypic variables for MG. In one study, the items in MG-QOL focusing on work skills were found to be less relevant for very late onset MG patients since the majority were retired (Zheng et al.). Once MG is well-controlled with immunotherapy, many patients stop pyridostigmine, may take it only when fatigued, or take 1∼2 tablets daily out of habit and for a sense of security (6). The influence of taking pyridostigmine on determination of the post-intervention status (PIS) categories was reported. In the same study, with a standardized flowchart and working definitions for the real-time and sustained (for 3, 6, and 12 months) PIS categories, sustainability of the R/MM status was confirmed in a prospective cohort of 376 patients with mild to moderate disease. The QMG, MG-ADL and MG-QOL15 scores among patients belonging to each real-time and sustained PIS category at baseline and follow-ups were significantly different, ranking as R < MM < SI. The GC and pyridostigmine doses decreased with time and ranked as R < MM < SI. This indicates that R/MM represents an immunologic stable state (Jiang et al.). Treatment response can be expressed as percentage of change from baseline (relative criterion) in autoimmune diseases (7). In a retrospective cohort of 257 immunotherapy-native MG patients, response to a 3-month standardized GC treatment was evaluated with commonly-used absolute criteria. Cut-offs for relative criteria were generated using a receiver-operating characteristic curves both for the whole cohort and in patients stratified

for pre-treatment QMG score. The consistency between the absolute criterion and the finally-selected relative criterion was substantial in the whole cohort, but was moderate in severe group. Some severe patients were classified as responsive with absolute criterion while as unresponsive with relative criterion. This finding is consistent with clinical experience (Li et al.).

Although evaluation of MG status by the clinicians is important in daily practice, patient-reported information and patient experience provide important knowledge on the disease itself and its management. MG research needs the input from patients who have experienced various symptoms, examinations and therapies, as well as multiple consequences of having MG. MG patients know from experience the needs for a precise diagnosis and better treatment, for correct information and more knowledge. The linguistic shift from "patient" to "user" reflects a change in ideology of medical research. The active participation of MG patients may bring something new into a research project, this also being true for subgroups such as children, pregnant women, the very old, and immigrants. In a thought-provoking opinion article, patient involvement was discussed in relevance to the phenotypic variation of MG (Gilhus et al.).

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to this editorial and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Suzuki S, Utsugisawa K, Suzuki N. Overlooked non-motor symptoms in myasthenia gravis. *J Neurol Neurosurg Psychiatry.* (2013) 84:989–94. doi: 10.1136/jnnp-2012-304057

2. Nacu A, Andersen JB, Lisnic V, Owe JF, Gilhus NE. Complicating autoimmune diseases in myasthenia gravis: a review. *Autoimmunity.* (2015) 48:362–68. doi: 10.3109/08916934.2015.1030614

3. Vogrig A, Muñiz-Castrillo S, Farina A, Honnorat J, Joubert B. How to diagnose and manage neurological toxicities of immune checkpoint inhibitors: an update. *J Neurol.* (2022) 269:1701–14. doi: 10.1007/s00415-021-10870-6

4. Narayanaswami P, Sanders DB, Wolfe G, Benatar M, Cea G, Evoli A, et al. International consensus guidance for management of myasthenia gravis: 2020 update. *Neurology.* (2021) 96:114–22. doi: 10.1212/WNL.0000000000011124

5. Morren J, Li Y. Myasthenia gravis with muscle-specific tyrosine kinase antibodies: a narrative review. *Muscle Nerve.* (2018) 58:344–58. doi: 10.1002/mus.26107

6. Dalakas MC. Immunotherapy in myasthenia gravis in the era of biologics. *Nat Rev Neurol.* (2019) 15:113–24. doi: 10.1038/s41582-018-0110-z

7. Quax RA, Koper JW, Huisman AM, Weel A, Hazes JM, Lamberts SW, et al. Polymorphisms in the glucocorticoid receptor gene and in the glucocorticoid-induced transcript 1 gene are associated with disease activity and response to glucocorticoid bridging therapy in rheumatoid arthritis. *Rheumatol Int.* (2015) 35:1325–33. doi: 10.1007/s00296-015-3235-z

frontiers in Neurology

ORIGINAL RESEARCH
published: 18 November 2021
doi: 10.3389/fneur.2021.767961

Patients With Myasthenia Gravis With Acute Onset of Dyspnea: Predictors of Progression to Myasthenic Crisis and Prognosis

*Yangyu Huang, Ying Tan, Jiayu Shi, Ke Li, Jingwen Yan and Yuzhou Guan**

Department of Neurology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China

Background: Life-threatening myasthenic crisis (MC) occurs in 10–20% of the patients with myasthenia gravis (MG). It is important to identify the predictors of progression to MC and prognosis in the patients with MG with acute exacerbations.

Objective: This study aimed to explore the predictors of progression to MC in the patients with MG with acute onset of dyspnea and their short-term and long-term prognosis.

Methods: This study is a retrospective cohort study. We collected and analyzed data on all the patients with MG with acute dyspnea over a 10-year period in a single center using the univariate and multivariate analysis.

Results: Eighty-six patients with MG were included. In their first acute dyspnea episodes, 36 (41.9%) episodes eventually progressed to MC. A multivariate analysis showed that the early-onset MG (adjusted *OR*: 3.079, 95% *CI* 1.052–9.012) and respiratory infection as a trigger (adjusted *OR*: 3.926, 95% *CI* 1.141–13.510) were independent risk factors for the progression to MC, while intravenous immunoglobulin (IVIg) treatment prior to the mechanical ventilation (adjusted *OR*: 0.253, 95% *CI* 0.087–0.732) was a protective factor. The prognosis did not significantly differ between the patients with and without MC during the MG course, with a total of 45 (52.3%) patients reaching post-intervention status better than minimal manifestations at the last follow-up.

Conclusion: When treating the patients with MG with acute dyspnea, the clinicians should be aware of the risk factors of progression to MC, such as early-onset MG and respiratory infection. IVIg is an effective treatment. With proper immunosuppressive therapy, this group of patients had an overall good long-term prognosis.

Keywords: myasthenia gravis, myasthenic crisis, impending myasthenic crisis, early-onset, intravenous immunoglobulin, mechanical ventilation, post-intervention status

OPEN ACCESS

Edited by:
Ghazala Hayat,
Saint Louis University, United States

Reviewed by:
Yuwei Da,
Capital Medical University, China
Marina Grandis,
University of Genoa, Italy

****Correspondence:***
Yuzhou Guan
guanyz001@163.com

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *31 August 2021*
Accepted: *18 October 2021*
Published: *18 November 2021*

Citation:
Huang Y, Tan Y, Shi J, Li K, Yan J and Guan Y (2021) Patients With Myasthenia Gravis With Acute Onset of Dyspnea: Predictors of Progression to Myasthenic Crisis and Prognosis. Front. Neurol. 12:767961. doi: 10.3389/fneur.2021.767961

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease caused by the autoantibodies that affect the structure of the post-synaptic membrane. Its main clinical manifestation is fluctuating muscle weakness (1). Myasthenic crisis (MC) refers to an event that requires mechanical ventilation because of severe involvement of the bulbar muscles or/and respiratory muscles (2). Furthermore, 10–20% of the patients with MG will develop MC (3–6), and most MC occurs within 2 years of the onset of MG (6, 7). IVIg and plasma exchange are recommended as the first-line therapy for impending and manifest myasthenic crisis (2, 8–10). However, the proportion of Chinese patients using IVIg and plasma exchange is significantly lower than that of the United States, and an Indian study on MC mentioned they treat the patients with MC with only corticosteroids and other immunosuppressive agents (11), suggesting that in the developing countries, many patients are not treated adequately due to the high price and unavailability of IVIg and plasma exchange. Therefore, exploring the risk factors of progress to MC in the patients with acute onset of dyspnea will aid clinical decision-making and medical resource allocation, especially in the developing countries. However, the previous studies focused on the clinical characteristics of the patients with MC (4, 12, 13) or the risk factors of MC occurrence after thymectomy (14–16), very few studies aimed to investigate the predictors of progression to MC in the patients with MG with acute onset of dyspnea. This study aimed to explore the predictors of progression to MC in the patients with MG with acute onset of dyspnea and their short-term and long-term prognosis.

METHODS

Study Design and Patient Selection

This study is a retrospective cohort study. We included all the patients with MG who complained of acute dyspnea and visited the emergency department of the Peking Union Medical College Hospital, Beijing, China from September 2010 to June 2020. The diagnosis of myasthenia gravis was made by satisfying the following three criteria: (1) fluctuating muscle weakness; (2) positive neostigmine test, or decrements of compound motor action potential showed in slow repetitive nerve stimulation, or positive serum anti-acetylcholine receptor (AChR) antibody; (3) other causes of skeletal muscle weakness excluded. The patients with dyspnea occurring within 4 weeks after thymectomy, or positive muscle-specific tyrosine kinase (MuSK) antibodies, or dyspnea because of acute exacerbation of chronic cardiopulmonary disease were excluded from this study. MC (2) was defined as receiving mechanical ventilation (non-invasive or invasive ventilation) or arterial blood gas analyses suggesting that the indications for mechanical ventilation were met ($PaCO_2 > 45$ mmHg and pH < 7.35, or oxygenation index $PaO_2/FiO_2 < 200$) (17). Multiple emergency department visits of the same patient were analyzed separately. We recommend IVIg for all the patients complaining of dyspnea. When mechanical ventilation was indicated, non-invasive ventilation was used first if the conditions permitted. All the patients were discharged from the hospital with oral corticosteroids and/or immunosuppressive agents. The final treatment choices were based on the informed consents of the patients and their family members. This study was approved by the Ethics Committee of Clinical Research of Peking Union Medical College Hospital (Beijing, China). The informed consents were obtained from every patient.

Data Acquisition

We collected data of all acute dyspnea episodes and selected the first episodes of each enrolled patient to explore the predictors of progression to MC. For predictive variables, we retrospectively collected demographic data, clinical features of MG, and comorbidities by reviewing the medical record data. All the patients underwent chest CT or contrast-enhanced chest CT, and the conditions of thymus were documented according to imaging or pathology. We obtained the data on the triggers of dyspnea, AChR antibody status, the Myasthenia Gravis Foundation of America (MGFA) clinical classification at the last follow-up before the episode, the treatments before the dyspnea episode, and the specific treatments (IVIg and glucocorticoid impulse therapy) used before the progression to crisis. For the outcome variables, the primary outcome was the occurrence of MC, the secondary outcomes included short-term prognosis and long-term prognosis, the former including the type and length of mechanical ventilation, total length of hospital stay, and in-hospital complications, and the long-term prognosis included the post-intervention status (PIS) at the last follow-up.

Statistics

The numerical variables were summarized as mean ± SD or median (inter quartile range, IQR), respectively, depending on whether they followed normal distribution. For univariate analysis, *t*-test or Wilcoxon's rank sum test was used for group comparisons of numerical variables, and chi-square test or Fisher's exact test was used for the categorical variables. Significance level α was set at 0.05 for both the sides. To find independent risk factors for progression to MC in the patients with acute onset of dyspnea, first dyspnea episodes of each patient were divided into two groups according to whether MC occurred, and the distribution of each predictor variable was compared between the two groups using a univariate analysis. All the variables that achieved $p < 0.10$ were selected for the bivariate logistic regression model. If multiple selected variables were correlated clinically (e.g., age of onset and early onset MG), only one variable of them was selected for the multivariate analysis. Thereafter, the *p*-values and adjusted *OR* values of the selected variables were calculated using the bivariate logistic regression. A principal component analysis was preformed to evaluate and eliminate multicollinearity. The data analysis was conducted using the IBM-SPSS (version 25, IBM, NY, USA).

RESULTS

Baseline Characteristics

We collected the clinical data from 86 patients with MG who had 111 visits to the emergency department for acute onset of dyspnea (**Table 1**). In total, 20 (23.3%) patients experienced

TABLE 1 | The baseline characteristics of first acute dyspnea episodes of the patients with myasthenia gravis (MG) and comparison between myasthenic crisis (MC) and non-MC episodes.

	All episodes (86 episodes)	MC episodes (36 episodes)	Non-MC episodes (50 episodes)	*p*-value
Female, *n* (%)	50 (58.1)	21 (58.3)	29 (58.0)	0.975
Thymoma, *n* (%)	35 (40.7)	17 (47.2)	18 (36.0)	0.296
Thymectomy, *n* (%)	23 (26.7)	11 (30.6)	12 (24.0)	0.498
Age of MG onset, mean ± SD	47.2 ± 17.8	41.1 ± 17.7	51.6 ± 16.7	0.006*
Early onset MG, *n* (%)	45 (52.3)	24 (66.7)	21 (42.0)	0.024*
AChR antibody status, *n* (%)				0.406
Positive	57 (66.3)	19 (52.8)	38 (76.0)	
Negative	7 (8.1)	4 (11.1)	3 (6.0)	
Unknown	22 (25.6)	13 (36.1)	9 (18.0)	
Involved muscle groups at MG onset				0.163
Ocular muscles, *n* (%)	62 (72.1)	23 (63.9)	39 (78.0)	
Limb muscles, *n* (%)	10 (11.6)	6 (16.7)	4 (8.0)	
Bulbar muscles, *n* (%)	12 (14.0)	5 (13.9)	7 (14.0)	
Cervical muscles, *n* (%)	2 (2.3)	2 (5.6)	0	
Respiratory muscles, *n* (%)	0	0	0	
Time from MG onset to dyspnea episode (month), median (IQR)	12.0 (4.0–34.5)	9.5 (4.3–33.0)	12.0 (2.0–37.5)	0.759
Age of dyspnea onset, mean ± SD or median (IQR)	51.0 ± 17.1	44.7 ± 17.0	60.5 (46.7–66.0)	0.004*
Involved muscle groups at dyspnea onset, *n* (%)	81 (94.2)	31 (86.1)	50 (100.0)	0.025*
Ocular muscles				
Limb muscles	66 (76.7)	27 (75.0)	39 (78.0)	0.745
Bulbar muscles	72 (83.7)	31 (86.1)	41 (82.0)	0.61
Cervical muscles	38 (44.2)	15 (41.7)	23 (46.0)	0.69
4 muscle groups involved	30 (34.9)	11 (30.6)	19 (38.0)	0.475
>2 muscle groups involved	60 (69.8)	23 (63.9)	37 (74.0)	0.314
MGFA classification at last visit before dyspnea episode, *n* (%)				0.024*
1	16 (18.6)	2 (5.6)	14 (28.0)	
2a	7 (8.1)	4 (11.1)	3 (6.0)	
2b	4 (4.7)	2 (5.6)	2 (4.0)	
3a	14 (16.3)	2 (5.6)	12 (24.0)	
3b	24 (27.9)	14 (38.9)	10 (20.0)	
4a	9 (10.5)	4 (11.1)	5 (10.0)	
4b	6 (7.0)	3 (8.3)	3 (6.0)	
Unknown	6 (7.0)	5 (13.9)	1 (2.0)	
MGFA classification worse than 3a, *n* (%)	53 (61.6)	23 (63.9)	30 (60.0)	0.714
Treatment before dyspnea episode, *n* (%)				0.603
No immunosuppressive treatments	58 (67.4)	23 (63.9)	35 (70.0)	
Steroids	19 (22.1)	8 (22.2)	11 (22.0)	
Other immunosuppressants alone	1 (1.2)	0	1 (2.0)	
Steroids and other immunosuppressants	8 (9.3)	5 (13.9)	3 (6.0)	
Respiratory infection as the trigger of the dyspnea episode, *n* (%)	21 (24.4)	13 (36.1)	8 (16.0)	0.037*
IVIg before MV, *n* (%)	55 (64.0)	17 (47.2)	38 (76.0)	0.006*

AChR, anti-acetylcholine receptor; IQR, inter quartile range; IVIg, intravenous immunoglobulin, MC, myasthenic crisis; MG, myasthenia gravis; MGFA classification, Myasthenia Gravis Foundation of America clinical classification; MV, mechanical ventilation. SD, standard deviation.
*$p < 0.05$.

multiple episodes of dyspnea (maximum of four episodes). Thirty-six (41.9%) of the patients were male. Of the 86 first dyspnea episodes of each patient, 35 (40.7%) occurred in the patients with thymoma and 23 (26.7%) in the patients who had undergone thymectomy. The mean age of MG onset was 47.2 ± 17.8 years, 45 (52.3%) dyspnea episodes occurred in the patients with early-onset MG (i.e., age of onset not older than 50), and 62 (72.1%) episodes occurred in the patients with MG with ocular onset. Fifty-seven (66.3%) patients had positive AChR antibody, while 22 (25.6%) patients lacked the data. The median time from MG onset to dyspnea episode was 12.0 (4.0–34.5) months, and the median age of dyspnea episode was 51.0 ± 17.1. We divided the skeletal muscles other than respiratory muscles into four groups: ocular muscles, limb muscles, bulbar muscles, and cervical muscles. Thirty (34.9%) dyspnea episodes occurred in the patients with involvement of all the four muscle groups, while 60 (69.8%) episodes in the patients with involvement of three or more groups. We also collected MGFA clinical classification at the last follow-up before the episode, and 53 (61.6%) dyspnea episodes occurred in the patients with MGFA clinical classification ≥3a at the last follow-up. Fifty-eight (67.4%) dyspnea episodes occurred in the patients who were not on corticosteroids or other immunosuppressive agents, while 56 (65.1%) episodes occurred in the patients who had not used corticosteroids before. Twenty-one (24.4%) episodes occurred after a trigger of respiratory infection, while other triggers included long-time fatigue, other infections, and other unspecified predisposing factors. In 55 (64.0%) dyspnea episodes, the patients received IVIg prior to mechanical ventilation. Regarding the comorbidities, the most frequent comorbidity was diabetes mellitus (14 episodes, 16.3%), followed by other autoimmune diseases (10 episodes, 11.6%). Thirty-six (41.9%) dyspnea episodes eventually progressed to MC, and 32 patients received mechanical ventilation, 28 (87.5%) of which ended with invasive mechanical ventilation.

Twenty (23.3%) patients experienced multiple episodes of dyspnea (maximum of four episodes), they had 45 episodes in total. Of the additional 25 episodes after the first episodes, 10 (40.0%) episodes were triggered by respiratory infection, 16 (64.0%) episodes were treated by IVIg. Ten (40.0%) episodes progressed to MC at last, similar to the first episode described above.

We analyzed the usage of IVIg at different time periods. From 2010 to 2013, IVIg was used in 20 out of 34 episodes (58.8%). This proportion was 36/57 (63.2%), 15/20 (75.0%) in different periods of 2014–2017 and 2018–2020, respectively. Although there appears to be an increasing in the use of IVIg, Wilcoxon's rank sum test did not reach statistical significance ($p = 0.484$).

Predictors of Progression to MC

Of the 86 first dyspnea episodes of each patient, 36 (41.9%) eventually progressed to MC (**Table 1**). By comparing the baseline characteristics between the MC group and non-MC group using the univariate analysis, we found that the variables with $p < 0.10$ included age of MG onset ($p = 0.006$), early-onset MG ($p = 0.024$), age at the dyspnea episode ($p = 0.009$), ocular muscle involvement when dyspnea episode occurred ($p = 0.025$), MGFA clinical classification at last follow-up ($p = 0.024$), respiratory infection as the trigger ($p = 0.037$), and IVIg therapy before mechanical ventilation ($p = 0.006$). Before including these variables in the binary logistic regression, we noted that age at onset correlated with early-onset or late-onset MG, and we took only early-onset MG into the regression. Thereafter, MGFA classification at last follow-up was dichotomized (≥3a vs. <3a).

TABLE 2 | The multivariable analysis for predictors of progression to MC.

Predictors	Adjusted OR	95% CI of adjusted OR	*p*-value
Early onset MG	3.079	1.052–9.012	0.040
Respiratory infection as a trigger	3.926	1.141–13.510	0.030
IVIg treatment prior to MV	0.253	0.087–0.732	0.011

IVIg, Intravenous immunoglobulin; MC, myasthenic crisis; MG, myasthenia gravis; MV, mechanical ventilation; OR, odds ratio.

The results of the bivariate logistic regression are shown in **Table 2**. The final equation suggested early-onset MG (adjusted *OR*: 3.079, 95% *CI* 1.052–9.012) and respiratory infection as a trigger (adjusted *OR*: 3.926, 95% *CI* 1.141–13.510) were independent risk factors for MC, while IVIg treatment prior to mechanical ventilation (adjusted *OR*: 0.253, 95% *CI* 0.087–0.732) was a protective factor for MC. Further the principal component analysis showed no covariance between the selected variables.

Clinical Characteristics and Prognosis of Patients With and Without MC During the MG Course

Of the 20 patients with multiple dyspnea episodes, seven patients did not experience MC in their first episodes but had MC in subsequent episodes. Thus, in all 86 patients, 49 (57.0%) had no MC during the course of MG, while 37 (43.0%) had MC (**Table 3**). The patients with early-onset MG were more likely to develop MC ($p = 0.025$). Thymoma was more frequent in the patients experienced MC, though the statistical difference was not significant (48.6 vs. 34.7%, $p = 0.192$). Follow-up time was defined as the time from the start of the last episode to the last follow-up visit. There was a significant difference in the follow-up time between the two groups ($p = 0.020$), with the patients without MC followed for 36.5 ± 22.8 months, while the patients with MC were followed for 60.4 ± 55.2 months. The long-term prognosis was similar in both the groups, with 57.1% of the non-MC group having PIS better than minimal manifestations (MM) at the last follow-up, compared with 45.9% of patients in the MC group.

Characteristics of Patients Requiring Invasive Ventilation Longer Than 15 Days

Of the 35 MC episodes requiring invasive ventilation, 12 (34.3%) had ventilation over 15 days (**Table 4**). One-third of them had comorbid diabetes mellitus, significantly more than the other group ($p = 0.038$). The patients requiring prolonged mechanical

TABLE 3 | The clinical characteristics and prognosis of patients with and without MC during the MG course.

	All patients (*n* = 86)	Non-MC patients (*n* = 49)	MC patients (*n* = 37)	*p*-value
Female, *n* (%)	50 (58.1)	30 (61.2)	20 (54.1)	0.517
Comorbidities, *n* (%)	5 (5.8)	3 (6.1)	2 (5.4)	0.684
Pulmonary disease				
Cardiac disease	2 (2.3)	1 (2.0)	1 (2.7)	1
Kidney disease	1 (1.2)	1 (2.0)	0	1
Liver disease	7 (8.1)	4 (8.2)	3 (8.1)	1
Diabetes mellitus	14 (16.3)	11 (22.4)	3 (8.1)	0.074
Neoplasm other than thymoma	3 (3.5)	2 (4.1)	1 (2.7)	1
Other autoimmune disease	10 (11.6)	5 (10.2)	5 (13.5)	0.893
Thymoma, *n* (%)	35 (40.7)	17 (34.7)	18 (48.6)	0.192
Thymectomy, *n* (%)	26 (30.2)	11 (22.4)	15 (40.5)	0.071
Age of MG onset, mean ± SD	47.2 ± 17.8	52.3 ± 16.1	40.4 ± 17.9	0.002
Early onset MG, *n* (%)	45 (52.3)	20 (40.8)	25 (67.6)	0.025
Involved muscle groups at dyspnea onset, *n* (%)				0.191
Ocular muscles	62 (72.1)	38 (77.6)	24 (64.9)	
Limb muscles	10 (11.6)	4 (8.2)	6 (16.2)	
Bulbar muscles	12 (14.0)	7 (14.3)	5 (13.5)	
Cervical muscles	2 (2.3)	0	2 (5.4)	
Respiratory muscles	0	0	0	
Follow-up time (month), mean ± SD or median (IQR)	41.0 (12.8–63.5)	36.5 ± 22.8	60.4 ± 55.2	0.02
PIS at last follow up, *n* (%)				0.307
CSR	2 (2.3)	1 (2.0)	1 (2.7)	
PR	34 (39.5)	23 (46.9)	11 (29.7)	
MM	9 (10.5)	4 (8.2)	5 (13.5)	
Improved	19 (22.1)	10 (20.4)	9 (24.3)	
Unchanged	1 (1.2)	1 (2.0)	0	
Exacerbation	11 (12.8)	5 (10.2)	6 (16.2)	
Death unrelated to MG	1 (1.2)	1 (2.0)	0	
Unknown	9 (10.5)	4 (8.2)	5 (13.5)	
PIS better than MM at last follow-up, *n* (%)	45 (52.3)	28 (57.1)	17 (45.9)	0.303

CSR, complete stable remission; IQR, inter quartile range; MC, myasthenic crisis; MG, myasthenia gravis; MM, minimal manifestations; PIS, Myasthenia Gravis Foundation of America (MGFA) post-intervention status; PR, Pharmacologic Remission; SD, standard variation.

ventilation also had significantly more pneumonia complications (83.3 vs. 39.1%, $p = 0.03$) and longer total length of hospital stay (46.5 vs. 25.7 days, $p = 0.003$). In addition, the patients requiring prolonged mechanical ventilation had older age (51.7 ± 13.1 years) than the other group (42.9 ± 20.2 years), but no significant difference was reached ($p = 0.13$).

DISCUSSION

In our present study, we demonstrated that in the patients with MG with acute onset of dyspnea, early-onset MG (adjusted *OR*: 3.079, 95% *CI* 1.052–9.012) and respiratory infection as a trigger (adjusted *OR*: 3.926, 95% *CI* 1.141–13.510) were independent risk factors for progression to MC, while the use of IVIg prior to mechanical ventilation (adjusted *OR*: 0.253, 95% *CI* 0.087–0.732) was a protective factor. The occurrence of MC had no significant impact on the long-term prognosis of these patients, and more than half of the patients (52.3%) reached a PIS better than MM at the last follow-up. Of the 35 MC episodes requiring invasive ventilation, 12 (34.3%) needed ventilation for more than 15 days. Comorbid diabetes mellitus (33.3 vs. 14.3%, $p = 0.038$) and complicated pneumonia (83.3 vs. 39.1%, $p = 0.030$) was associated with prolonged ventilation.

We found that early-onset MG was an independent risk factor for progression to MC in the patients with MG with acute onset of dyspnea (**Table 2**). Similar to our findings, A. Ramos-Fransi et al. (7) found that in the patients with MG with life-threatening events (i.e., MGFA class V or class IVB), early-onset MG had a longer time to weaning from ventilation, suggesting that early-onset MG may have more severe life-threatening events and worse response to the treatment. Since most patients in our study had positive AChR antibodies and the positive rate was similar between the MC and non-MC episodes, the reason for early-onset MG was a risk factor may be that in the patients with

TABLE 4 | The characteristics of patients requiring invasive ventilation longer than 15 days.

	Invasive ventilation (*n* = 35)	Ventilation < 15 days (*n* = 23)	Ventilation > 15 days (*n* = 12)	*p*-value
Female, *n* (%)	18 (51.4)	14 (60.9)	4 (33.3)	0.164
Comorbidities, *n* (%)	3 (8.6)	2 (8.3)	1 (8.3)	1
Pulmonary disease				
Cardiac disease	1 (2.9)	1 (4.3)	0	1
Kidney disease	0	0	0	-
Liver disease	5 (14.3)	3 (13.0)	2 (16.7)	1
Diabetes mellitus	5 (14.3)	1 (4.3)	4 (33.3)	0.038
Neoplasm other than thymoma	0	0	0	-
Other autoimmune disease	6 (17.1)	3 (13.0)	3 (25.0)	0.391
Thymoma, *n* (%)	19 (54.3)	13 (56.5)	6 (50.0)	0.736
Thymectomy, *n* (%)	13 (37.1)	9 (39.1)	4 (33.3)	0.164
Age of MG onset, mean ± SD or median (IQR)	39.6 ± 18.6	31.0 (21.0–55.0)	44.4 ± 18.2	0.203
Early onset MG, *n* (%)	23 (65.7)	15 (65.2)	8 (66.7)	1
Age of dyspnea onset, mean ± SD	45.9 ± 18.3	42.9 ± 20.2	51.7 ± 13.1	0.13
Respiratory infection as the trigger of the dyspnea episode, *n* (%)	16 (45.7)	11 (47.8)	5 (41.7)	1
IVIg before MV, *n* (%)	15 (42.9)	9 (39.1)	6 (50.0)	0.721
Length of hospital stay (day), mean ± SD or median (IQR)	29.0 (7.0-560.0)	25.7 ± 14.0	46.5 (30.5-72.0)	0.003
Complications, *n* (%)	19 (54.3)	9 (39.1)	10 (83.3)	0.03
Pneumonia				
Liver injury	5 (14.3)	4 (17.4)	1 (8.3)	0.64
Kidney injury	2 (5.7)	1 (4.3)	1 (8.3)	1
Cardiac arrest	2 (5.7)	0	2 (16.7)	0.111
Infection other than pneumonia	4 (11.4)	2 (8.7)	2 (16.7)	0.594

IQR, inter quartile range; IVIg, intravenous immunoglobulin; MG, myasthenia gravis; MV, mechanical ventilation; SD, standard variation.

positive AChR antibodies, early-onset MG and late-onset MG are thought to differ significantly in pathogenesis. The origin of autoimmune antibodies in early-onset MG is mostly in the thymus, while the role of the thymus in the pathogenesis of late-onset MG is unclear, since no detectable inflammation was found in the thymus (1). Besides, the clinical characteristics of early-onset MG differ from of late-onset MG, one of which is that the response of early-onset MG to immunotherapy and the prognosis are worse, which may be associated with an expansion in protective immunomodulatory mechanisms, such as peripheral T-regulatory cells, in the elderly (18).

The previous studies have shown that infection is the most common trigger of MC as well as other life-threatening events in the patients with MG (3, 4, 7, 12, 19, 20). Further, our study also found that among the patients with MG with acute onset of dyspnea, respiratory infection was an independent risk factor for progression to MC. Infection may lead to exacerbation of the symptoms of bulbar palsy and respiratory muscle weakness, while the respiratory infections can aggravate ventilation dysfunction and lead to impaired air exchange, increasing the probability of mechanical ventilation. For the patients with MG with acute dyspnea with respiratory infection, close monitoring of vital signs and preparation for mechanical ventilation is particularly essential.

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus and has become a global pandemic (21). Although there were no patients with COVID-19 in our cases, the effect of COVID-19 on the patients with MG is noteworthy in the context of pandemic. Dyspnea caused by SARS-CoV-2 infection may have a greater risk of progression to MC, as we discussed above. The patients with MG may be more vulnerable to COVID-19 due to the immunosuppressive therapies (22). However, the current evidence on the relationship between MG and COVID-19 is highly variable. Some studies suggested that COVID-19 has a limited effect on most of the patients with MG, since most of the patients in these studies do not experience exacerbations of MG, and immunosuppressive therapy is relatively safe (23–25). In contrast, other studies found a high proportion of patients with MG exacerbations requiring rescue therapy or mechanical ventilation (26, 27). High-quality studies on the relationship between COVID-19 and MC are also lacking, which may be explained by the difficulty in diagnosing MC in patients with COVID-19. Besides MC, severe COVID-19 may also lead to respiratory failure. Furthermore, many previous cases used drugs such as hydroxychloroquine and azithromycin that may exacerbate the MG symptoms or cause myopathy, further complicating the relationship between respiratory

failure and MC. Further well-designed, and prospective studies are needed.

The efficacy of IVIg in the patients with MG with acute exacerbation has been demonstrated in several randomized controlled trials (8). Likewise, our study found that IVIg was a protective factor for progression to MC in the patients with acute onset of dyspnea. Since IVIg and plasma exchange are equally effective in worsening MG (9), and IVIg is easier to use, our center uses IVIg to treat this group of patients. Although the international consensus guidance (2) and multiple national guidelines (10, 28) recommended IVIg and plasma exchange as first-line therapy for impending and manifest myasthenic crisis, IVIg was not used timely in 31.0% of acute dyspnea episodes in this study. Based on our experience, we speculate that the reason for this is that IVIg is still expensive for the patients with MG in the developing countries, even when health insurance can partially cover the cost, which prevents some patients with MG with acute dyspnea from receiving timely treatment.

All the patients in our study were prescribed with oral corticosteroids and/or immunosuppressant agents after discharge. The overall prognosis was good, with 52.3% of patients having a PIS better than MM at last follow-up, with a median follow-up time of 41.0 months. There was no significant difference in the PIS at the last follow-up between the patients experienced MC and those who did not, which is similar to other studies. Sivadasan, A et al. (29) studied the patients with MC admitted to the intensive care unit. All the patients received oral corticosteroids and immunosuppressant agents and were followed for a median time of 36 months, with 67% of them reaching PIS better than MM at the last follow-up. Spillane, J et al. (20) studied the patients with MG admitted to the intensive care unit due to acute exacerbation. Most of the patients (97%) were on oral steroids and nearly half (45%) were started with other immunosuppressants. At a median follow-up time of 4 years, 19% of patients were asymptomatic at the last follow-up and 48% reached MGFA classification better than type II. Both these studies and our study suggest that the long-term use of immunosuppressive therapy after the acute phase significantly improves the long-term prognosis of patients with MG who experienced acute dyspnea.

Our study found that in the patients requiring invasive ventilation longer than 15 days, a significantly higher proportion of patients had comorbid diabetes mellitus, prolonged length of hospital stay, and complications of pneumonia. The age at onset of dyspnea was also older in this group, although it did not reach significance difference (51.7 ± 13.1 vs. 42.9 ± 20.2, $p = 0.13$). Similar to other studies, advanced age and more chronic underlying diseases were risk factors for prolonged mechanical ventilation (3, 12), suggesting that this group of patients may require better intensive care unit management and have a higher probability of tracheotomy.

Of the patients included in this study, 67.4% had not used immunosuppressive therapy, such as oral steroids or immunosuppressants before their first dyspnea episodes. Similarly, the proportion of this group of patients in other studies was 40–50% (3, 20). Several studies showed that early immunosuppressive treatment of MG reduces the probability of MC occurrence and recurrence (30). To date, several retrospective studies showed that the use of oral steroids in ocular MG could reduce the probability of progression to generalized MG (31, 32). These results suggest that the use of immunosuppressive therapy in the patients with MG may not only improve symptoms, but also act as a disease modifier to prevent the progression of the disease course in the patients with MG.

The greatest limitation of this study arises from its single-center retrospective nature. The relative rarity of MG and the low incidence of respiratory or bulbar muscle involvement in MG resulted in a small sample size, so the results may be incidental. Selection bias is inevitable in single center studies. The lower incidence of comorbidities and the higher incidence of thymoma in our patients compared with other studies may limit the generalization of the findings to all the patients. However, compared with other studies that focused mostly on the patients with MG who had already developed MC, we expanded the target population to include the patients with MG with acute onset of dyspnea (i.e., the patients may progress to MC), effectively expanded the sample size and increased the credibility of the final conclusions.

The previous studies have focused on the patients with manifest MC, and most studies have been conducted in intensive care unit settings (4, 12, 13). A strength of this study is that we described the clinical characteristics of impending the patients with MC from the emergency department, which makes our study more relevant to the clinical practice of neurologists. Nowadays, many patients with MG with acute dyspnea in the developing countries are unable to receive IVIg because of its high price and unavailability. The significance of this study is that we explored the risk factors for progression to MC in this group of patients, indicating that when treating the patients with MG with acute dyspnea, the clinicians should be more aggressive in advancing the use of IVIg to avoid MC occurrence in early-onset MG or dyspnea triggered by respiratory infection. Initiating social assistance or transferring the patients to a qualified center are effective options.

In conclusion, we demonstrated that early-onset MG and respiratory infection as a trigger were the independent risk factors for progression to MC in the patients with MG with acute onset of dyspnea, while the use of IVIg prior to mechanical ventilation was a protective factor. With proper immunosuppressive therapy, this group of patients had an overall good prognosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Clinical Research of Peking

Union Medical College Hospital (Beijing, China). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YH and YG contributed to conception and design of the study. YH, YT, JS, JY, and KL collected the clinical data. YH, YT, and KL contributed to the data analysis and interpretation. YH wrote the manuscript. YT and YG edited the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by the National Clinical Cohort Study of Rare Diseases for key R & D Program (grant number: 2016YFC091501) and the Beijing Municipal Sciences & Technology Commission (Z181100001718145).

REFERENCES

1. Gilhus NE, Tzartos S, Evoli A, Palace J, Burns TM, Verschuuren JJGM. Myasthenia gravis. *Nat Rev Dis Prim.* (2019) 5:30. doi: 10.1038/s41572-019-0079-y
2. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. *Neurology.* (2016) 87:419–25. doi: 10.1212/WNL.0000000000002790
3. Thomas CE, Mayer SA, Gungor Y, Swarup R, Webster EA, Chang I, et al. Myasthenic crisis: clinical features, mortality, complications, and risk factors for prolonged intubation. *Neurology.* (1997) 48:1253–60. doi: 10.1212/WNL.48.5.1253
4. Sakaguchi H, Yamashita S, Hirano T, Nakajima M, Kimura E, Maeda Y, et al. Myasthenic crisis patients who require intensive care unit management. *Muscle Nerve.* (2012) 46:440–2. doi: 10.1002/mus.23445
5. Alshekhlee A, Miles JD, Katirji B, Preston DC, Kaminski HJ. Incidence and mortality rates of myasthenia gravis and myasthenic crisis in US hospitals. *Neurology.* (2009) 72:1548–54. doi: 10.1212/WNL.0b013e3181a41211
6. Cohen MS, Younger D. Aspects of the natural history of myasthenia gravis: crisis and death. *Ann N Y Acad Sci.* (1981) 377:670–7. doi: 10.1111/j.1749-6632.1981.tb33765.x
7. Ramos-Fransi A, Rojas-Garcia R, Segovia S, Marquez-Infante C, Pardo J, Coll-Canti J, et al. Myasthenia gravis: descriptive analysis of life-threatening events in a recent nationwide registry. *Eur J Neurol.* (2015) 22:1056–61. doi: 10.1111/ene.12703
8. Gajdos P, Chevret S, Toyka KV. Intravenous immunoglobulin for myasthenia gravis. *Cochrane Database Syst Rev.* (2012) 12:CD002277. doi: 10.1002/14651858.CD002277.pub4
9. Dhawan PS, Goodman BP, Harper CM, Bosch PE, Hoffman-Snyder CR, Wellik KE, et al. IVIG versus PLEX in the treatment of worsening myasthenia gravis: what is the evidence?: a critically appraised topic. *Neurologist.* (2015) 19:145–8. doi: 10.1097/NRL.0000000000000026
10. Evoli A, Antonini G, Antozzi C, DiMuzio A, Habetswallner F, Iani C, et al. Italian recommendations for the diagnosis and treatment of myasthenia gravis. *Neurol Sci.* (2019) 40:1111–24. doi: 10.1007/s10072-019-03746-1
11. Kalita J, Kohat AK, Misra UK. Predictors of outcome of myasthenic crisis. *Neurol Sci.* (2014) 35:1109–14. doi: 10.1007/s10072-014-1659-y
12. Neumann B, Angstwurm K, Mergenthaler P, Kohler S, Schonenberger S, Bosel J, et al. Myasthenic crisis demanding mechanical ventilation: a multicenter analysis of 250 cases. *Neurology.* (2020) 94:e299–313. doi: 10.1212/WNL.0000000000008688
13. Souayah N, Nasar A, Suri MF, Kirmani JF, Ezzeddine MA, Qureshi AI. Trends in outcomes and hospitalization charges among mechanically ventilated patients with myasthenia gravis in the United States. *Int J Biomed Sci.* (2009) 5:209–14. Available online at: http://www.ijbs.org/User/ContentFullText.aspx?VolumeNO=5&StartPage=209
14. Tian W, Li X, Tong H, Weng W, Yang F, Jiang G, et al. Surgical effect and prognostic factors of myasthenia gravis with thymomas. *Thorac Cancer.* (2020) 11:1288–96. doi: 10.1111/1759-7714.13396
15. Choi KH, Nam TS, Lee SH, Kim MK. Preoperative pulmonary function is strongly related to myasthenic crisis after thymectomy. *Neurol India.* (2014) 62:164–8. doi: 10.4103/0028-3886.132361
16. Ando T, Omasa M, Kondo T, Yamada T, Sato M, Menju T, et al. Predictive factors of myasthenic crisis after extended thymectomy for patients with myasthenia gravis. *Eur J Cardiothorac Surg.* (2015) 48:705–9; discussion 9. doi: 10.1093/ejcts/ezu530
17. Nava S, Hill N. Non-invasive ventilation in acute respiratory failure. *Lancet.* (2009) 374:250–9. doi: 10.1016/S0140-6736(09)60496-7
18. Cortes-Vicente E, Alvarez-Velasco R, Segovia S, Paradas C, Casasnovas C, Guerrero-Sola A, et al. Clinical and therapeutic features of myasthenia gravis in adults based on age at onset. *Neurology.* (2020) 94:e1171–80. doi: 10.1212/WNL.0000000000008903
19. Roper J, Fleming ME, Long B, Koyfman A. Myasthenia gravis and crisis: evaluation and management in the emergency department. *J Emerg Med.* (2017) 53:843–53. doi: 10.1016/j.jemermed.2017.06.009
20. Spillane J, Hirsch NP, Kullmann DM, Taylor C, Howard RS. Myasthenia gravis–treatment of acute severe exacerbations in the intensive care unit results in a favourable long-term prognosis. *Eur J Neurol.* (2014) 21:171–3. doi: 10.1111/ene.12115
21. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol.* (2021) 19:141–54. doi: 10.1038/s41579-020-00459-7
22. Jacob S, Muppidi S, Guidon A, Guptill J, Hehir M, Howard JF, Jr., et al. Guidance for the management of myasthenia gravis (MG) and Lambert-Eaton myasthenic syndrome (LEMS) during the COVID-19 pandemic. *J Neurol Sci.* (2020) 412:116803. doi: 10.1016/j.jns.2020.116803
23. Jakubíková M, Týblová M, Tesar A, Horáková M, VlaŽná D, Ryšánková I, et al. Predictive factors for a severe course of COVID-19 infection in myasthenia gravis patients with an overall impact on myasthenic outcome status and survival. *Eur J Neurol.* (2021) 28:3418–25. doi: 10.1111/ene.14951
24. Solé G, Mathis S, Friedman D, Salort-Campana E, Tard C, Bouhour F, et al. Impact of coronavirus disease 2019 in a French cohort of myasthenia gravis. *Neurology.* (2021) 96:e2109–20. doi: 10.1212/WNL.0000000000011669
25. Businaro P, Vaghi G, Marchioni E, Diamanti L, Arceri S, Bini P, et al. COVID-19 in patients with myasthenia gravis: epidemiology and disease course. *Muscle Nerve.* (2021) 64:206–11. doi: 10.1002/mus.27324
26. Camelo-Filho AE, Silva AMS, Estephan EP, Zambon AA, Mendonça RH, Souza PVS, et al. Myasthenia gravis and COVID-19: clinical characteristics and outcomes. *Front Neurol.* (2020) 11:1053. doi: 10.3389/fneur.2020.01053
27. Muppidi S, Guptill JT, Jacob S, Li Y, Farrugia ME, Guidon AC, et al. COVID-19-associated risks and effects in myasthenia gravis (CARE-MG). *Lancet Neurol.* (2020) 19:970–1. doi: 10.1016/S1474-4422(20)30413-0
28. Sussman J, Farrugia ME, Maddison P, Hill M, Leite MI, Hilton-Jones D. Myasthenia gravis: association of British neurologists' management guidelines. *Pract Neurol.* (2015) 15:199–206. doi: 10.1136/practneurol-2015-001126
29. Sivadasan A, Alexander M, Aaron S, Mathew V, Nair S, Muthusamy K, et al. Comorbidities and long-term outcomes in a cohort with myasthenic crisis:

experiences from a tertiary care center. *Ann Indian Acad Neurol.* (2019) 22:464–71. doi: 10.4103/aian.AIAN_197_19
30. Juel VC. Myasthenic crisis: smoother sailing ahead. *Eur J Neurol.* (2009) 16:775–6. doi: 10.1111/j.1468-1331.2009.02629.x
31. Wong SH, Huda S, Vincent A, Plant GT. Ocular myasthenia gravis: controversies and updates. *Curr Neurol Neurosci Rep.* (2014) 14:421. doi: 10.1007/s11910-013-0421-9
32. Aguirre F, Villa AM. Prognosis of ocular myasthenia gravis in an argentinian population. *Eur Neurol.* (2018) 79:113–7. doi: 10.1159/000487132

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Neurology

ORIGINAL RESEARCH
published: 22 November 2021
doi: 10.3389/fneur.2021.790941

Comorbid Autoimmune Diseases in Patients With Myasthenia Gravis: A Retrospective Cross-Sectional Study of a Chinese Cohort

Jianquan Shi[1], *Xiao Huan*[2,3], *Lei Zhou*[2,3], *Jianying Xi*[2,3], *Jie Song*[2,3], *Ying Wang*[4], *Sushan Luo*[2,3]* *and Chongbo Zhao*[2,3]*

[1] *Department of Neurology, Nanjing First Hospital, Nanjing Medical University, Nanjing, China,* [2] *Department of Neurology, Huashan Hospital Fudan University, Shanghai, China,* [3] *National Center for Neurological Disorders, Shanghai, China,* [4] *Department of Pharmacy, Huashan Hospital, Fudan University, Shanghai, China*

Introduction: The phenomenon of coexisting autoimmune diseases (ADs) in patients with myasthenia gravis (MG) has attracted considerable attention. However, few studies have investigated the burden and potential clinical associations of ADs in Chinese MG cohorts.

Methods: In this retrospective cross-sectional study, we reviewed the records of 1,132 patients with MG who were admitted to Huashan Hospital Fudan University from August 2013 to August 2020. Patients were excluded if they had incomplete medical records ($n = 336$).

Results: Comorbid ADs were found in 92 of 796 Chinese patients with MG (11.6%), among which, hyperthyroidism (6.7%), hypothyrosis (2.6%), and vitiligo (0.8%) were predominant. Patients with MG with ADs were predominantly female, younger at the onset of MG symptoms, and had a lower frequency of thymoma. Compared to the general population, we found a significantly higher percentage of hyperthyroidism (8.5-fold increase, $p < 0.001$), hypothyrosis (2.6-fold increase, $p < 0.001$), vitiligo (1.3-fold increase, $p < 0.001$), rheumatoid arthritis (1.4-fold increase, $p < 0.001$), immune thrombocytopenic purpura (193.1-fold increase, $p < 0.001$), autoimmune hemolytic anemia (7.4-fold increase, $p < 0.001$), autoimmune hepatitis (5.1-fold increase, $p < 0.001$), and polymyositis (11.5-fold increase, $p < 0.001$) in patients with MG with ADs. Patients with MG with ADs presented a lower proportion of previous history of MC (0 vs. 5.6%, $p < 0.05$) than those without ADs. The proportion of MGFA Class I at onset in patients with MG with ADs was significantly higher than that in patients with MG without ADs (77.0 vs. 52.7%, $p < 0.05$). The proportion of MuSK-positive in patients with MG with ADs was significantly lower than that in patients with MG without ADs (0 vs. 4.8%, $p < 0.05$).

Conclusion: In conclusion, we observed a higher frequency of concurrent ADs in a Chinese MG cohort. Furthermore, MG combined with ADs tended to have mild clinical presentation.

Keywords: myasthenia gravis, autoimmune diseases, comorbidities, percentage, clinical characteristics

OPEN ACCESS

Edited by:
Nils Erik Gilhus, University of Bergen, Norway

Reviewed by:
Jose Flores, Manuel Velasco Suárez Instituto Nacional de Neurología y Neurocirugía, Mexico
Carmelo Rodolico, University of Messina, Italy

***Correspondence:**
Chongbo Zhao
zhao_chongbo@fudan.edu.cn
Sushan Luo
luosushan@fudan.edu.cn

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *07 October 2021*
Accepted: *01 November 2021*
Published: *22 November 2021*

Citation:
Shi J, Huan X, Zhou L, Xi J, Song J, Wang Y, Luo S and Zhao C (2021) Comorbid Autoimmune Diseases in Patients With Myasthenia Gravis: A Retrospective Cross-Sectional Study of a Chinese Cohort. Front. Neurol. 12:790941. doi: 10.3389/fneur.2021.790941

INTRODUCTION

Myasthenia gravis (MG) is a rare autoimmune disorder of the neuromuscular junction, which is characterized by fatigable weakness in extraocular muscles, limbs, and even bulbar muscles (1). The disorder is typically mediated by antibodies against acetylcholine receptor (AChR) or other proteins located at the neuromuscular junction, including muscle-specific tyrosine kinase (MuSK) and lipoprotein receptor-related protein 4 (2). The incidence of MG ranges from 0.3 to 2.8 per 100,000 worldwide, which varies with age, sex, and ethnic groups (3, 4).

Similar to other autoimmune diseases (ADs), genetic factors contribute to the susceptibility of developing MG (5). Mounting evidence has demonstrated common genetic signals in many ADs, suggesting the possibility of shared common pathogenetic mechanisms (6). Several studies from western countries and Japan have suggested that ∼13% of patients with MG also have other ADs (7–10). The most common AD in patients with MG is autoimmune thyroid disease, followed by systemic lupus erythematosus and rheumatoid arthritis (11). MG in association with ADs often has mild clinical expression (11). However, it remains unclear whether there are clinically significant differences in Chinese patients with MG with and without ADs. Therefore, we investigated the percentages of comorbid ADs in a Chinese MG cohort. Furthermore, we compared the clinical characteristics between patients with MG with and without ADs.

METHODS

Study Design and Patient Recruitment

Through our single-center registration database, we reviewed the records of all patients with MG who were admitted to Huashan Hospital Fudan University from August 2013 to August 2020. The diagnosis of MG was based on clinical symptoms and at least one of the following specific tests: objective clinical response to neostigmine test, seropositivity for anti-AChR/anti-MuSK antibody, or significant decremental response on 3 Hz repetitive nerve stimulation (12, 13). Other diseases that mimic MG were excluded, including Lambert-Eaton syndrome, motor neuron disease, and congenital myasthenic syndrome.

ADs were identified if they were included in the list defined by Hayter and Cook, with the addition of psoriasis (14–16). Diagnoses of ADs were based on the clinical manifestations, laboratory test (including specific antibodies), biopsy results (if required), and reference to the diagnostic criteria of ADs by the corresponding specialists.

This retrospective cohort study was approved by the Institutional Review Board of Huashan Hospital Fudan University. Written informed consent was obtained from each study participant.

Abbreviations: MG, Myasthenia gravis; AChR, acetylcholine receptor; MuSK, muscle-specific tyrosine kinase; ADs, autoimmune diseases; MC, myasthenic crisis; MGFA, Myasthenia Gravis Foundation of America; ITP, Immune thrombocytopenic purpura; AHA, Autoimmune hemolytic anemia; AH, Autoimmune hepatitis.

Data Collection

Clinical data were collected from the recruited patients, including age at onset, duration, sex, thymoma concurrence, thymectomy, history of myasthenic crisis (MC), family history of ADs, history of allergic diseases, family history of allergic diseases, history of malignancies, Myasthenia Gravis Foundation of America (MGFA) Class at onset, serum antibody status, and the presence of comorbid ADs.

Statistical Analysis

Statistical analyses were performed using IBM SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). The normality of continuous variables was tested by Shapiro–Wilks test. Continuous variables that followed a normal distribution are presented as the mean ± standard deviation. Categorical variables are expressed as frequencies (percentages). The chi-square test for specified proportions was used to compare the percentages of various ADs in patients with MG to the corresponding prevalence in the general population. The most recent prevalence data for each AD were selected for comparison. Data obtained from the Chinese population were used when available. The prevalence of other countries was used when Chinese prevalence data were unavailable. Independent sample *t*-test and chi-square test were used to compare the differences between the two groups as appropriate (MG with ADs vs. MG without ADs; MG with 1 AD vs. MG with ≥2 ADs). A two-tailed $p < 0.05$ was considered significant.

RESULTS

Clinical Characteristics of MG Cohort

The database comprised 1,132 steroid or immunosuppressant naïve patients who were diagnosed with MG during the study period. Patients were excluded if they had incomplete medical records ($n = 336$). Ultimately, 796 patients with MG were enrolled in the study, including 704 patients without ADs and 92 patients with ADs (84 with 1 ADs and 8 with ≥2 ADs) (**Figure 1**).

The clinical characteristics of patients with MG are presented (**Table 1**). Among the 796 patients, 454 (57.0%) were female, the average age at onset was 40.4 ± 18.1 years, and the average duration was 2.9 ± 5.6 years. Thymoma was present in 214 cases (26.9%), and thymectomy was performed in 120 cases (15.1%). As the initial symptom, ocular weakness alone (MGFA Class I) occurred in 56.4% (449/796) of patients, and 43.5% (346/796) of patients were classified into generalized type (MGFA Class II, III, IV). Only 4.3% (34/796) of patients suffered from MC. Two hundred and sixty-one cases (32.8%) had no information on antibody test. Approximately 84.9% (454/535) of patients were AChR-positive, 4.1% (22/535) of patients were MuSK-positive, and 11.0% (59/535) of patients were double-seronegative.

Types and Percentages of Comorbid ADs in MG Cohort

Ninety-two patients (59 females and 33 females, 11.6%) with MG had ≥1 comorbid ADs. The frequencies of overall ADs in our cohort were higher than the background prevalence of the population (total patients: 11.6 vs. 5.0%,

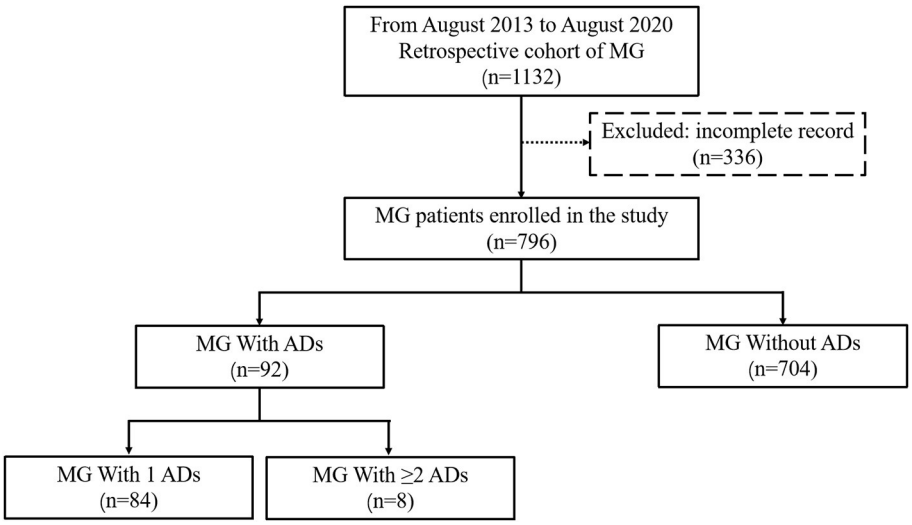


FIGURE 1 | Flow diagram of the study design. A total of 796 patients with MG were enrolled in the study, including 704 patients with MG without ADs and 92 with ADs (84 with 1 ADs and 8 with ≥2 ADs). MG, Myasthenia gravis; Ads, Autoimmune diseases.

TABLE 1 | Clinical characteristics of patients with MG.

	No. of cases (%) (*n* = 796)
Age at onset (years)	40.4 ± 18.1
Duration (years)	2.9 ± 5.6
Sex	
Male	342 (43.0%)
Female	454 (57.0%)
Age at onset (years)	
<50	537 (67.5%)
≥50	259 (32.5%)
Thymoma concurrence	
No	582 (73.1%)
Yes	214 (26.9%)
Thymectomy	120 (15.1%)
History of MC	
No	762 (95.7%)
Yes	34 (4.3%)
MGFA at onset	
I	449 (56.4%)
II	318 (39.9%)
III	25 (3.1%)
IV	3 (0.4%)
Unknown	1 (0.1%)
Antibody status	
AChR-positive	454 (57.0%)
MuSK-positive	22 (2.8%)
Seronegative	59 (7.4%)
Unknown	261 (32.8%)
Antibody status (*n* = 535)	
AChR-positive	454 (84.9%)
MuSK-positive	22 (4.1%)
Seronegative	59 (11.0%)

MG, Myasthenia gravis; MC, Myasthenic crisis; MGFA, Myasthenia Gravis Foundation of America; AChR, Acetylcholine receptors; MuSK, Muscle-specific tyrosine kinase.

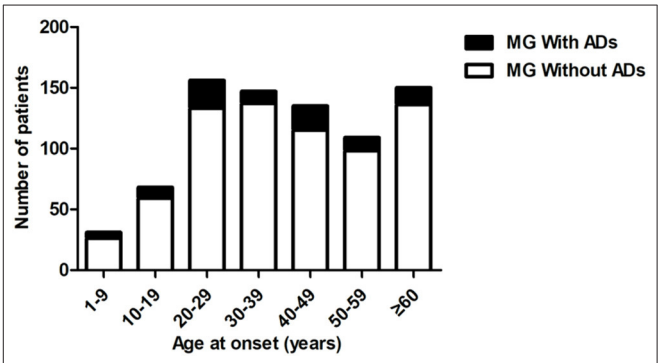


FIGURE 2 | Percentage of comorbid ADs by age at onset. ADs, Autoimmune diseases.

male patients: 9.6 vs. 3.0%, female patients: 13.0 vs. 7.1%) (15). The percentages of comorbid ADs varied among different onset age groups (from 6.8 to 16.1%, **Figure 2**). The percentages of comorbid ADs in patients with early onset

TABLE 2 | Comorbid ADs in patients with MG.

	Frequency (%) ($n = 796$)	Cases (extrapolated to 100,000 patients with MG)	Prevalence in the general population (region)
Hyperthyroidism	53 (6.7%)	6658	0.78% (China) (17)
Hypothyrosis	21 (2.6%)	2638	1.02% (China) (17)
Vitiligo	6 (0.8%)	754	0.56% (China) (18)
Hashimoto's thyroiditis	5 (0.6%)	628	1.7% (China) (19)
Rheumatoid arthritis	4 (0.5%)	503	0.37% (China) (20)
Sjögren's syndrome	4 (0.5%)	503	0.45% (China) (20)
Psoriasis	2 (0.3%)	251	0.47% (China) (21)
ITP	2 (0.3%)	251	0.0013% (France) (22)
AHA	1 (0.1%)	126	0.017% (Denmark) (23)
Autoimmune hepatitis	1 (0.1%)	126	0.0245% (New Zealand) (24)
Polymyositis	1 (0.1%)	126	0.011% (Africa) (25)

MG, Myasthenia gravis; ADs, Autoimmune diseases; ITP, Immune thrombocytopenic purpura; AHA, Autoimmune hemolytic anemia.

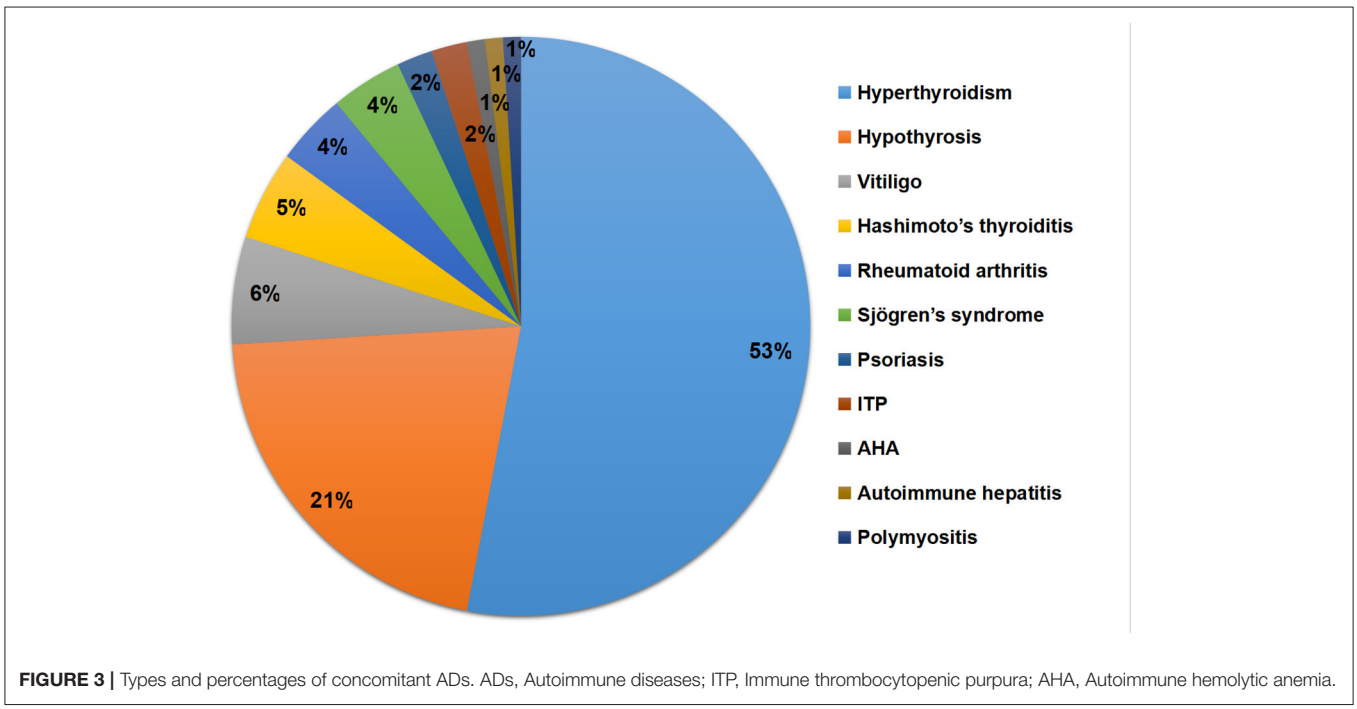


FIGURE 3 | Types and percentages of concomitant ADs. ADs, Autoimmune diseases; ITP, Immune thrombocytopenic purpura; AHA, Autoimmune hemolytic anemia.

MG (67/537, 12.5%) were higher than those in patients with late-onset MG (25/259, 9.7%). The percentage of comorbid ADs in patients with MG without thymoma (75/582, 12.9%) was higher than that in patients with MG with thymoma (17/214, 7.9%).

The types, frequencies, and percentages of concomitant ADs are presented in **Table 2** and **Figure 3**. Thyroid disease was the most common comorbid AD, observed in 79 (9.9%) patients with MG. The percentage of hyperthyroidism, hypothyrosis, and Hashimoto's thyroiditis was 6.7% ($n = 53$), 2.6% ($n = 21$), and 0.6% ($n = 5$), respectively. Other types of ADs included vitiligo ($n = 6$, 0.8%), rheumatoid arthritis ($n = 4$, 0.5%), Sjögren's syndrome ($n = 4$, 0.5%), psoriasis ($n = 2$, 0.3%), idiopathic thrombocytopenic purpura ($n = 2$, 0.3%), autoimmune hemolytic anemia ($n = 1$, 0.1%), autoimmune hepatitis ($n = 1$, 0.1%), and polymyositis ($n = 1$, 0.1%).

The percentage of each observed AD in our cohort and the prevalence of the disease in the general population are presented in **Table 2**. The percentage of each observed AD was extrapolated to 100,000 patients with MG and compared to the prevalence of the disease in the general population (**Table 2** and **Figure 4**). We found a significantly higher percentage of hyperthyroidism (8.5-fold increase, $p < 0.001$) (17), hypothyrosis (2.6-fold increase, $p < 0.001$) (17), vitiligo (1.3-fold increase, $p < 0.001$) (18), rheumatoid arthritis (1.4-fold increase, $p < 0.001$) (20), immune thrombocytopenic purpura (193.1-fold increase, $p < 0.001$) (22), autoimmune hemolytic anemia (7.4-fold increase, $p < 0.001$) (23), autoimmune hepatitis (5.1-fold increase, $p <$

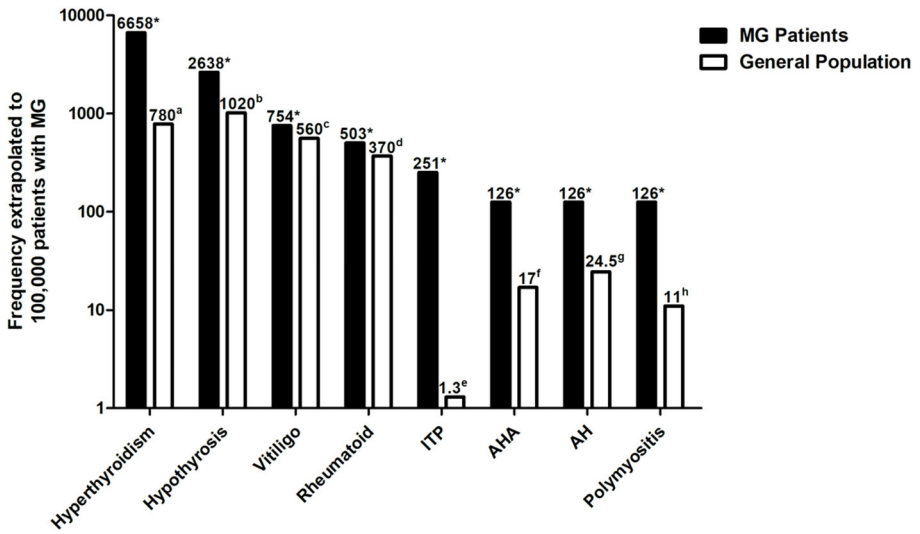


FIGURE 4 | Percentage of each observed AD was extrapolated to 100,000 patients with MG and compared to the prevalence of the disease in the general population. Autoimmune diseases with a significantly higher percentage in patients with MG compared to the general population are shown. The Y-axis is in logarithmic scale. [a]Data derived from Li et al. (17); [b]data derived from Li et al. (17); [c]data derived from Wang et al. (18); [d]data derived from Xiang et al. (20); [e]data derived from Mariotte et al. (22); [f]data derived from Hansen et al. (23); [g]data derived from Ngu et al. (24); [h]data derived from Essouma et al. (25); *$p < 0.05$ vs. the prevalence of the disease in the general population. ADs, Autoimmune diseases; MG, Myasthenia gravis.

0.001) (24), and polymyositis (11.5-fold increase, $p < 0.001$) (25). We observed at a higher percentage of Sjögren's syndrome in patients with MG (20), although the difference did not reach statistical significance (1.1-fold increase, $p = 0.091$). Hashimoto's thyroiditis and psoriasis were observed at a lower prevalence in patients with MG (19, 21).

Clinical Characteristics Between MG With and Without ADs

We compared the clinical characteristics between patients with MG with and without ADs. Patients with MG with ADs presented a lower proportion of previous history of MC (0 vs. 4.8%, $p < 0.05$) and a higher proportion of family history of ADs (20.7 vs. 4.5%, $p < 0.05$) than those without ADs. The proportion of MGFA Class I at onset in patients with MG with ADs was significantly higher than that in those without ADs (72.8 vs. 54.3%, $p < 0.05$). No significant differences in other clinical features were found between patients with MG with and without ADs ($p > 0.05$) (**Supplementary Table 1**).

We next compared the clinical characteristics between patients with MG with and without ADs for whom antibody information was available. Patients with MG with ADs presented a lower proportion of previous history of MC (0 vs. 5.6%, $p < 0.05$) and a higher proportion of family history of ADs (21.6 vs. 5.4%, $p < 0.05$) than those without ADs. The proportion of MGFA Class I at onset in patients with MG with ADs was significantly higher than that in patients with MG without ADs (77.0 vs. 52.7%, $p < 0.05$). The proportion of MuSK-positive in patients with MG with ADs was significantly lower than that in patients with MG without ADs (0 vs. 4.8%, $p < 0.05$). No significant differences in other clinical features were found between patients with MG with and without ADs for whom antibody information was available ($p > 0.05$) (**Table 3**).

Clinical Characteristics Between Patients With MG With 1 and ≥2 ADs

We next compared the clinical characteristics between patients with MG with 1 and ≥2 ADs. No significant differences in clinical features were found between patients with MG with 1 and ≥2 ADs ($p > 0.05$) (**Supplementary Table 2**).

DISCUSSION

This study revealed that the percentages of some ADs in our cohort were higher than the background prevalence. Furthermore, patients with MG with ADs presented a lower proportion of previous history of MC. The proportion of MGFA Class I at onset in patients with MG with ADs was significantly higher than that in patients with MG without ADs. The proportion of MuSK-positive in patients with MG with ADs was significantly lower than that in patients with MG without ADs.

It has been demonstrated that MG often coexists with ADs in European or Japanese cohorts. The percentages of concomitant ADs in Norwegian and Danish cohorts have been shown to be 22.9 and 9.4%, respectively (7, 8). A Japanese cohort study reported that associated ADs were found in 28 of 142 patients with MG (19.7%) (9). Moreover, a Swedish population-based study suggested that patients with MG had an increased risk of another AD compared to controls (22.0 vs. 8.9%) (26). A systematic review revealed that the pooled estimate of coexisting ADs in MG was 13% (10). However, few studies have been conducted in Chinese cohorts to date. The percentage of overall

TABLE 3 | Clinical characteristics between patients with MG with and without ADs for whom antibody information were available.

	MG with ADs ($n = 74$)	MG without ADs ($n = 461$)	p
Age at onset (years)	38.7 ± 17.7	40.7 ± 17.9	0.367
Duration (years)	3.7 ± 6.4	2.7 ± 5.0	0.175
Sex			0.077
Male	25 (33.8%)	209 (45.3%)	
Female	49 (66.2%)	252 (54.7%)	
Age at onset (years)			0.504
<50	53 (71.6%)	310 (67.2%)	
≥50	21 (28.4%)	151 (32.8%)	
Thymoma concurrence			0.204
No	59 (79.7%)	333 (72.2%)	
Yes	15 (20.3%)	128 (27.8%)	
Thymectomy			0.299
No	66 (89.2%)	387 (83.9%)	
Yes	8 (10.8%)	74 (16.1%)	
History of MC			0.037*
No	74 (100%)	435 (94.4%)	
Yes	0 (0%)	26 (5.6%)	
Family history of ADs			0.000*
No	58 (78.4%)	436 (94.6%)	
Yes	16 (21.6%)	25 (5.4%)	
History of allergic diseases			0.437
No	68 (91.9%)	434 (94.1%)	
Yes	6 (8.1%)	27 (5.9)	
Family history of allergic diseases			0.698
No	72 (97.3%)	450 (97.6%)	
Yes	2 (2.7%)	11 (2.4%)	
History of malignancies			1
No	72 (97.3%)	446 (96.7%)	
Yes	2 (2.7%)	15 (3.3%)	
MGFA at onset			0.001*
I	57 (77.0%)	243 (52.7%)	
II	17 (23.0%)	195 (42.3%)	
III	0 (0%)	19 (4.1%)	
IV	0 (0%)	3 (0.7%)	
Unknown	0 (0%)	1 (0.2%)	
Antibody status			0.022*
AChR-positive	63 (85.1%)	391 (84.8%)	
MuSK-positive	0 (0%)	22 (4.8%)	
Seronegative	11 (14.9%)	48 (10.4%)	

*MG, Myasthenia gravis; ADs, Autoimmune diseases; MC, Myasthenic crisis; MGFA, Myasthenia Gravis Foundation of America; AChR, Acetylcholine receptor; MuSK, Muscle-specific tyrosine kinase. *means p value is < 0.05.*

ADs in our cohort was 11.6%, which was similar to previous studies. The frequency varies widely across studies, which might be due to the differences in study populations, the ascertainment criteria, and selection bias. Increasing evidence from analysis of human leukocyte antigen haplotypes and genome-wide association studies has revealed shared etiopathogenic factors in many ADs (27). However, the exact mechanisms of comorbid ADs in MG are elusive and require further investigation.

The results of this study suggested that the clinical features of patients with MG with ADs were predominantly female and were younger at onset of MG symptoms. Similar to our findings, studies from Danish, Japanese, and Swedish cohorts also suggested a stronger association among younger and female patients with MG (8, 9, 15). Thymoma-associated MG has an unexpected pattern regarding coexisting ADs. Previous studies have reported a lower percentage of second ADs in thymoma-associated MG than that in non-thymoma-associated MG (28–30). A lower risk for autoimmune comorbidity has also been demonstrated in patients with muscle antibodies against titin and ryanodine receptor, which predicts the presence of thymoma in patients with MG (11, 31, 32). Similarly, we found that the percentage of comorbid ADs in patients with MG with thymoma was lower than that in those without thymoma. However, patients with thymic hyperplasia had a higher frequency of concomitant ADs than those without thymic hyperplasia (9). Few studies have reported the frequency of associated ADs in MuSK-positive MG. We found that MuSK-positive MG was rarely accompanied by ADs; this may be ascribed to the rare occurrence of thymic hyperplasia in MuSK-positive MG, which is highly associated with ADs (33). Due to the limitation of small numbers of MusK-MG patients, more cases should be involved to identify it in future study.

In this study, the most commonly associated conditions in patients with MG were hyperthyroidism and hypothyrosis, both of which were present at a significantly increased percentage compared to the general population. A meta-analysis was performed to determine the prevalence of thyroid disorders in patients with MG, which included 39 studies with 24,927 patients with MG (34). The results suggested that the prevalence of thyroid disorders, hyperthyroidism, and hypothyrosis in patients with MG was 10.1, 5.6, and 2.6%, respectively, which was similar to our results. However, the meta-analysis showed that the prevalence of Hashimoto's thyroiditis in patients with MG was 4.6%, which was significantly higher than 0.6% of our cohort. The lower rate of Hashimoto's thyroiditis in our cohort might be ascribed to the differences in demographic variations and the bias resulting from the cross-sectional nature of this study. A common genetic background may explain the high frequency of autoimmune thyroid disease in MG, and human leukocyte antigens B8 and DR3 have been reported in both disorders (35, 36).

Although thyroid disease has been established as a MG-associated comorbidity, vitiligo has been less frequently reported. We observed a 1.3-fold increase in the frequency of vitiligo in patients with MG compared to the general population. Cruz et al. reported that the percentage of vitiligo in a MG cohort was <1.7% (1 in over 60 patients with MG) (37). Moreover, Kubota et al. found a low frequency (0.5%) of vitiligo in patients with MG (38). Compared to the aforementioned studies, our results are in the middle of the previously observed range. Gill et al. found a significantly higher prevalence of MG (0.2%, 36-fold increase compared to the general population) in a cohort of 1,873 patients with vitiligo (16). A 10-year cross-sectional

retrospective study of an urban US population also suggested a significantly higher prevalence of MG in 1,487 patients with vitiligo (39). The higher percentage of vitiligo in patients with MG (or the higher prevalence of MG in patients with vitiligo) suggests that these diseases share a similar underlying pathogenesis. Our results support associations between MG and rheumatoid arthritis or polymyositis, which have also been found in other MG cohorts (11, 40). Our study evidences several new comorbid ADs in patients with MG, including immune thrombocytopenic purpura, autoimmune hemolytic anemia, and autoimmune hepatitis. Although concomitant presentation of these diseases with MG has been reported in rare cases, to our knowledge, these diseases have never been investigated in large cohorts of patients with MG. Although no case was found in the present cohort, it is worth noting that several reports indicated MG may coexist with neuromyelitis optica spectrum disorder (41–43). Previous case reports were mostly complicated with thymoma or thymectomy, indicating an immunological link between the central nervous system and the muscles might exist and be partially due to immune dysregulation or paraneoplastic mechanism. Although aquaporin 4 protein was found to harbor in thymus, the underlying precise mechanism is not clearly understood (44).

In this study, patients with MG with ADs showed a higher frequency of ocular MG at onset. Similarly, the results from an Italian cohort and a Polish cohort suggested that MG associated with autoimmune thyroid diseases had a mild clinical expression, with preferential ocular involvement (27, 45). Ocular MG has a unique link to thyroid disease, which might be ascribed to immunological cross-reactivity against epitopes or autoantigens shared by the thyroid and eye muscles (28, 46, 47). Furthermore, we found that patients with MG with ADs were less susceptible to MC. Moreover, our findings suggested that patients with MG combined with ADs tended to have mild clinical presentation, which is consistent with other studies (28, 29).

This study has some limitations. First, bias is inevitable given that the study is a retrospective single-center study. Second, the study is limited by the lack of longitudinal follow-up data. Therefore, a prospective, multiple-center, follow-up study should be conducted to study comorbid ADs in MG.

CONCLUSIONS

In conclusion, we observed a higher frequency of concurrent ADs in a Chinese MG cohort. Thyroid disease was the most common comorbid AD, while other types of ADs included vitiligo, rheumatoid arthritis, Sjögren's syndrome, psoriasis, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, autoimmune hepatitis, and polymyositis. Patients with MG with ADs were predominantly female, younger at the onset of MG symptoms, and had a lower frequency of thymoma. Furthermore, MG combined with ADs tended to have mild clinical presentation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Huashan Hospital Fudan University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JS and XH contributed to statistical analysis and drafting of the manuscript. SL and CZ conducted the study design and modified the manuscript. LZ, JX, JS, and YW contributed in collection and validation of the clinical and laboratory data.

FUNDING

This work was supported by financial grants from the National Natural Science Foundation of China (Nos. 81803542, 81870988, and 82071410), Shanghai Municipal Science and Technology Major Project (No. 2018SHZDZX01), and ZJLab.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.790941/full#supplementary-material

REFERENCES

1. Gilhus NE. Myasthenia gravis. *N Engl J Med*. (2016) 375:2570–81. doi: 10.1056/NEJMra1602678
2. Spillane J, Higham E, Kullmann DM. Myasthenia gravis. *BMJ*. (2012) 345:e8497. doi: 10.1136/bmj.e8497
3. Meriggioli MN, Sanders DB. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. *Lancet Neurol*. (2009) 8:475–90. doi: 10.1016/S1474-4422(09)70063-8
4. Dresser L, Wlodarski R, Rezania K, Soliven B. Myasthenia gravis: epidemiology, pathophysiology and clinical manifestations. *J Clin Med*. (2021) 10:2235. doi: 10.3390/jcm10112235
5. Zhong H, Zhao C, Luo S. HLA in myasthenia gravis: from superficial correlation to underlying mechanism. *Autoimmun Rev*. (2019) 18:102349. doi: 10.1016/j.autrev.2019.102349
6. Voight BF, Cotsapas C. Human genetics offers an emerging picture of common pathways and mechanisms in autoimmunity. *Curr Opin Immunol*. (2012) 24:552–7. doi: 10.1016/j.coi.2012.07.013

7. Thorlacius S, Aarli JA, Riise T, Matre R, Johnsen HJ. Associated disorders in myasthenia gravis: autoimmune diseases and their relation to thymectomy. *Acta Neurol Scand*. (1989) 80:290–5. doi: 10.1111/j.1600-0404.1989.tb03881.x
8. Christensen PB, Jensen TS, Tsiropoulos I, Sørensen T, Kjaer M, Højer-Pedersen E, et al. Associated autoimmune diseases in myasthenia gravis. A population-based study. *Acta Neurol Scand*. (1995) 91:192–5. doi: 10.1111/j.1600-0404.1995.tb00432.x
9. Kanazawa M, Shimohata T, Tanaka K, Nishizawa M. Clinical features of patients with myasthenia gravis associated with autoimmune diseases. *Eur J Neurol*. (2007) 14:1403–4. doi: 10.1111/j.1468-1331.2007.01978.x
10. Mao ZF, Yang LX, Mo XA, Qin C, Lai YR, He NY, et al. Frequency of autoimmune diseases in myasthenia gravis: a systematic review. *Int J Neurosci*. (2011) 121:121–9. doi: 10.3109/00207454.2010.539307
11. Gilhus NE, Nacu A, Andersen JB, Owe JF. Myasthenia gravis and risks for comorbidity. *Eur J Neurol*. (2015) 22:17–23. doi: 10.1111/ene.12599
12. Mantegazza R, Cavalcante P. Diagnosis and treatment of myasthenia gravis. *Curr Opin Rheumatol*. (2019) 31:623–33. doi: 10.1097/BOR.0000000000000647
13. Huan X, Luo S, Zhong H, Zheng X, Song J, Zhou L, et al. In-depth peripheral CD4+ T profile correlates with myasthenic crisis. *Ann Clin Transl Neurol*. (2021) 8:749–62. doi: 10.1002/acn3.51312
14. Di Cesare A, Di Meglio P, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. *J Invest Dermatol*. (2009) 129:1339–50. doi: 10.1038/jid.2009.59
15. Hayter SM, Cook MC. Updated assessment of the prevalence, spectrum and case definition of autoimmune disease. *Autoimmun Rev*. (2012) 11:754–65. doi: 10.1016/j.autrev.2012.02.001
16. Gill L, Zarbo A, Isedeh P, Jacobsen G, Lim HW, Hamzavi I. Comorbid autoimmune diseases in patients with vitiligo: a cross-sectional study. *J Am Acad Dermatol*. (2016) 74:295–302. doi: 10.1016/j.jaad.2015.08.063
17. Li Y, Teng D, Ba J, Chen B, Du J, He L, et al. Efficacy and safety of long-term universal salt iodization on thyroid disorders: epidemiological evidence from 31 provinces of Mainland China. *Thyroid*. (2020) 30:568–79. doi: 10.1089/thy.2019.0067
18. Wang X, Du J, Wang T, Zhou C, Shen Y, Ding X, et al. Prevalence and clinical profile of vitiligo in China: a community-based study in six cities. *Acta Derm Venereol*. (2013) 93:62–5. doi: 10.2340/00015555-1397
19. Teng W, Shan Z, Teng X, Guan H, Li Y, Teng D, et al. Effect of iodine intake on thyroid diseases in China. *N Engl J Med*. (2006) 354:2783–93. doi: 10.1056/NEJMoa054022
20. Xiang YJ, Dai SM. Prevalence of rheumatic diseases and disability in China. *Rheumatol Int*. (2009) 29:481–90. doi: 10.1007/s00296-008-0809-z
21. Ding X, Wang T, Shen Y, Wang X, Zhou C, Tian S, et al. Prevalence of psoriasis in China: a population-based study in six cities. *Eur J Dermatol*. (2012) 22:663–7. doi: 10.1684/ejd.2012.1802
22. Mariotte E, Azoulay E, Galicier L, Rondeau E, Zouiti F, Boisseau P, et al. Epidemiology and pathophysiology of adulthood-onset thrombotic microangiopathy with severe ADAMTS13 deficiency (thrombotic thrombocytopenic purpura): a cross-sectional analysis of the French national registry for thrombotic microangiopathy. *Lancet Haematol*. (2016) 3:e237–45. doi: 10.1016/S2352-3026(16)30018-7
23. Hansen DL, Möller S, Andersen K, Gaist D, Frederiksen H. Increasing incidence and prevalence of acquired hemolytic anemias in Denmark, 1980–2016. *Clin Epidemiol*. (2020) 12:497–508. doi: 10.2147/CLEP.S250250
24. Ngu JH, Bechly K, Chapman BA, Burt MJ, Barclay ML, Gearry RB, et al. Population-based epidemiology study of autoimmune hepatitis: a disease of older women? *J Gastroenterol Hepatol*. (2010) 25:1681–6. doi: 10.1111/j.1440-1746.2010.06384.x
25. Essouma M, Noubiap JJ, Singwe-Ngandeu M, Hachulla E. Epidemiology of idiopathic inflammatory myopathies in Africa: a contemporary systematic review [published online ahead of print, 2021 Apr 9]. *J Clin Rheumatol*. (2021). doi: 10.1097/RHU.0000000000001736
26. Fang F, Sveinsson O, Thormar G, Granqvist M, Askling J, Lundberg IE, et al. The autoimmune spectrum of myasthenia gravis: a Swedish population-based study. *J Intern Med*. (2015) 277:594–604. doi: 10.1111/joim.12310
27. Parkes M, Cortes A, van Heel DA, Brown MA. Genetic insights into common pathways and complex relationships among immune-mediated diseases. *Nat Rev Genet*. (2013) 14:661–73. doi: 10.1038/nrg3502
28. Marinó M, Ricciardi R, Pinchera A, Barbesino G, Manetti L, Chiovato L, et al. Mild clinical expression of myasthenia gravis associated with autoimmune thyroid diseases. *J Clin Endocrinol Metab*. (1997) 82:438–43. doi: 10.1210/jc.82.2.438
29. Chen YL, Yeh JH, Chiu HC. Clinical features of myasthenia gravis patients with autoimmune thyroid disease in Taiwan. *Acta Neurol Scand*. (2013) 127:170–4. doi: 10.1111/j.1600-0404.2012.01693.x
30. Nacu A, Andersen JB, Lisnic V, Owe JF, Gilhus NE. Complicating autoimmune diseases in myasthenia gravis: a review. Autoimmunity. (2015) 48:362–8. doi: 10.3109/08916934.2015.1030614
31. Cordts I, Bodart N, Hartmann K, Karagiorgou K, Tzartos JS, Mei L, et al. Screening for lipoprotein receptor-related protein 4-, agrin-, and titin-antibodies and exploring the autoimmune spectrum in myasthenia gravis. *J Neurol*. (2017) 264:1193–203. doi: 10.1007/s00415-017-8514-z
32. Lazaridis K, Tzartos SJ. Myasthenia gravis: autoantibody specificities and their role in MG management. *Front Neurol*. (2020) 11:596981. doi: 10.3389/fneur.2020.596981
33. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol*. (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3
34. Song RH, Yao QM, Wang B, Li Q, Jia X, Zhang JA. Thyroid disorders in patients with myasthenia gravis: a systematic review and meta-analysis. *Autoimmun Rev*. (2019) 18:102368. doi: 10.1016/j.autrev.2019.102368
35. Kraemer MH, Donadi EA, Tambascia MA, Magna LA, Prigenzi LS. Relationship between HLA antigens and infectious agents in contributing towards the development of graves' disease. *Immunol Invest*. (1998) 27:17–29. doi: 10.3109/08820139809070887
36. Giraud M, Beaurain G, Yamamoto AM, Eymard B, Tranchant C, Gajdos P, et al. Linkage of HLA to myasthenia gravis and genetic heterogeneity depending on anti-titin antibodies. *Neurology*. (2001) 57:1555–60. doi: 10.1212/WNL.57.9.1555
37. Cruz MW, Maranhão Filho PA, André C, Mattos JP, Novis SA. Myasthenia gravis and vitiligo. *Muscle Nerve*. (1994) 17:559–60. doi: 10.1002/mus.880170519
38. Kubota A, Komiyama A, Tanigawa A, Hasegawa O. Frequency and clinical correlates of vitiligo in myasthenia gravis. *J Neurol*. (1997) 244:388–9. doi: 10.1007/s004150050107
39. Hadi A, Wang JF, Uppal P, Penn LA, Elbuluk N. Comorbid diseases of vitiligo: a 10-year cross-sectional retrospective study of an urban US population. *J Am Acad Dermatol*. (2020) 82:628–33. doi: 10.1016/j.jaad.2019.07.036
40. Garibaldi M, Fionda L, Vanoli F, Leonardi L, Loreti S, Bucci E, et al. Muscle involvement in myasthenia gravis: expanding the clinical spectrum of myasthenia-myositis association from a large cohort of patients. *Autoimmun Rev*. (2020) 19:102498. doi: 10.1016/j.autrev.2020.102498
41. Spillane J, Christofi G, Sidle KC, Kullmann DM, Howard RS. Myasthenia gravis and neuromyelitis opica: a causal link. *Mult Scler Relat Disord*. (2013) 2:233–7. doi: 10.1016/j.msard.2013.01.003
42. Bibic VC, Brust TB, Burton JM. Neuromyelitis optica spectrum disorder presenting with concurrent autoimmune diseases. *Mult Scler Relat Disord*. (2019) 28:125–8. doi: 10.1016/j.msard.2018.12.028
43. Castro-Suarez S, Guevara-Silva E, Caparó-Zamalloa C, Cortez J, Meza-Vega M. Neuromyelitis optica in patients with myasthenia gravis: two case-reports. *Mult Scler Relat Disord*. (2020) 43:102173. doi: 10.1016/j.msard.2020.102173
44. Vaknin-Dembinsky A, Abramsky O, Petrou P, Ben-Hur T, Gotkine M, Brill L, et al. Myasthenia gravis-associated neuromyelitis optica-like disease: an immunological link between the central nervous system and muscle? *Arch Neurol*. (2011) 68:1557–61.doi: 10.1001/archneurol.2011.200
45. Kubiszewska J, Szyluk B, Szczudlik P, Bartoszewicz Z, Dutkiewicz M, Bielecki M, et al. Prevalence and impact of autoimmune thyroid disease on myasthenia gravis course. *Brain Behav*. (2016) 6:e00537. doi: 10.1002/brb3.537
46. Ludgate M, Swillens S, Mercken L, Vassart G. Homology between thyroglobulin and acetylcholinesterase: an explanation for pathogenesis of Graves' ophthalmopathy? *Lancet*. (1986) 2:219–20. doi: 10.1016/S0140-6736(86)92515-8

47. Zimmermann CW, Eblen F. Repertoires of autoantibodies against homologous eye muscle in ocular and generalized myasthenia gravis differ. *Clin Investig.* (1993) 71:445–51. doi: 10.1007/BF00180057

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Neurology

ORIGINAL RESEARCH
published: 18 January 2022
doi: 10.3389/fneur.2021.809999

Real-World Healthcare Resource Utilization and Cost Burden Assessment for Adults With Generalized Myasthenia Gravis in the United States

Glenn Phillips[1], Catarina Abreu[2], Amit Goyal[3], Yuebing Li[4], Albert Whangbo[2], Deborah Gelinas[1], Edward Brauer[1] and Sankha Bhattacharya[5]*

[1] argenx US Inc., Boston, MA, United States, [2] ZS Associates, New York, NY, United States, [3] ZS Associates, Princeton, NJ, United States, [4] Neuromuscular Center, Cleveland Clinic, Cleveland, OH, United States, [5] ZS Associates, Bangalore, India

OPEN ACCESS

Edited by:
Hai-Feng Li,
Capital Medical University, China

Reviewed by:
Raghav Govindarajan,
University of Missouri, United States
Henry Kaminski,
George Washington University, United States

***Correspondence:**
Glenn Phillips
gphillips@argenx.com

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *05 November 2021*
Accepted: *21 December 2021*
Published: *18 January 2022*

Citation:
Phillips G, Abreu C, Goyal A, Li Y, Whangbo A, Gelinas D, Brauer E and Bhattacharya S (2022) Real-World Healthcare Resource Utilization and Cost Burden Assessment for Adults With Generalized Myasthenia Gravis in the United States. Front. Neurol. 12:809999. doi: 10.3389/fneur.2021.809999

Introduction: Limited evidence exists for healthcare resource utilization (HCRU) and costs associated with generalized myasthenia gravis (gMG), a rare autoimmune disorder, for adults in the United States.

Methods: Adults with ≥1 diagnostic claim for MG between 2014 and 2019 were identified using Symphony Health's Integrated Dataverse®. Using a novel algorithm, HCRU and costs over 12 months following index dates were evaluated for patients with gMG including those with exacerbation events. For patients who experienced crisis events, HCRU and costs were analyzed during the 36 months preceding, during, and 12 months following the events.

Results: Mean HCRU and costs were higher for newly diagnosed patients compared with previously diagnosed patients (hospitalizations: 0.46 vs. 0.34; all-cause costs: $26,419.20 vs. $24,941.47; direct costs for gMG treatments: $9,890.37 vs. $9,186.47) and further increased for patients with exacerbation events (hospitalizations: 0.72; all-cause costs: $43,734.15; direct costs for gMG treatments: $21,550.02). For patients who experienced crisis events, HCRU and costs markedly increased during the 12 months immediately before the crisis event (hospitalizations: 1.35; all-cause costs: $49,236.68) compared with the 2 preceding years and increased further during the 12 months following the crisis index date (hospitalizations: 2.78; all-cause costs: $173,956.99). Cost increases were, in large part, attributed to treatments received.

Discussion: New diagnosis, exacerbation, and crisis events were drivers of HCRU and cost for patients with gMG. Particularly, high costs of gMG-specific medications associated with intervention for exacerbation and crisis events contributed to increased all-cause costs.

Keywords: myasthenia gravis (MG), myasthenic crisis, healthcare resource utilization (HCRU), cost analysis, disease burden

INTRODUCTION

Myasthenia gravis (MG) is a rare autoimmune disorder associated with the failure of neuromuscular junction (NMJ) transmission and is characterized by autoantibodies that target specific proteins involved in NMJ signaling (1). The most common autoantigen is the acetylcholine receptor; other autoantigens involved in NMJ formation and maintenance (e.g., MuSK [muscle-specific kinase] and LRP4 [low-density lipoprotein receptor-related protein 4]) have been identified in a subset of patients (1). In the United States (US), prevalence is estimated at 14–20 per 100,000 population with ∼60,000 patients currently living with MG, though figures are likely higher since MG is often underdiagnosed (2–4). While MG presents with ocular symptoms in approximately two-thirds of patients, symptoms remain isolated to the ocular area in only 15% of cases (referred to as ocular MG or oMG) (1). For the remaining majority of patients, the disease progresses to generalized MG (gMG), which may involve bulbar, limb, trunk, and respiratory muscles (5). Exacerbation of gMG symptoms can lead to a myasthenic crisis defined as respiratory failure requiring mechanical ventilation, which occurs at least once in 15% to 20% of patients with MG during their lifetime (6).

A systematic literature review of the economic burden of MG in the US showed that healthcare resource utilization (HCRU) associated with MG was particularly increased in patients with treatment-refractory MG (defined as receiving multiple or a complex regimen of MG therapies) and patients experiencing crisis events (7–9). However, limited US-based evidence was available for cost burden (10), and available studies were outdated for providing reliable cost estimates—particularly, the cost burden of gMG has not been clearly delineated from that of oMG (11). With the objective of filling essential knowledge gaps, we evaluated HCRU and costs associated with gMG (including myasthenic crisis management) by designing a novel analysis method validated by gMG experts based on data extracted from a comprehensive US claims database. As a secondary objective, we evaluated treatment patterns associated with subgroups of patients with gMG.

MATERIALS AND METHODS

Expert Interviews

Five US-based neuromuscular specialists experienced in gMG management were interviewed individually during 1-h sessions on disease epidemiology, treatment patterns, and clinical practice of gMG. All experts reviewed the initial approaches for the cost analysis, provided input on the methodology, and were engaged throughout the analysis for relevant feedback. Further details on the interview structure can be found in the **Supplementary Methods**.

Study Design and Data Source

This retrospective observational study was conducted using Symphony Health's (Blue Bell, PA, USA) Integrated Dataverse (IDV®) of pharmaceutical and medical claims. The IDV® claims database links healthcare data of ∼280 million enrollees in the US from pharmacy point-of-service sales, within-network transactions, and additional direct prescriptions (including medical and hospital claims data feeds). The robust database captures a high proportion of prescription transactions across the US and includes information from a range of payment types including commercial insurance, Medicare, Medicaid, and cash. At the authors' request, Symphony Health (Blue Bell, PA, USA) identified study patients spanning the period of January 1, 2014 to December 31, 2019, and relevant data were licensed by authors through ZS Associates' (Evanston, IL, USA) data partnership agreement with Symphony Health (Blue Bell, PA, USA). The de-identified claims data included details on patient prescriptions and diagnoses as well as procedural, surgical, and health service data, and the settings in which they were administered. NDCs (National Drug Codes) and CPT® (Current Procedural Terminology®) codes were used to identify therapies, services, and procedures received by patients across outpatient, inpatient, and other care settings from the extracted dataset. No identifiable or protected health information was obtained for use in this study.

Patient Selection

Inclusion Criteria

Adults (≥18 years of age) with claims including International Classification of Diseases, Ninth or Tenth Editions (ICD-9 or ICD-10) diagnostic codes associated with MG were included in the initial screening (**Supplementary Table 1**). Patients with ≥2 such claims filed at least 1 month apart between January 1, 2014 and December 31, 2019 were selected to limit cases involving misdiagnoses. Further, patients with MG diagnostic claims filed only by ophthalmologic specialists (defined as ophthalmologists, pediatric ophthalmologists, or optometrists) were considered more likely to be diagnosed with oMG instead of gMG and were excluded from the analysis. The final study cohort included patients who had at least 1 claim filed between January 1, 2017 and December 31, 2018 (**Supplementary Figure 1**).

Subgroup Definitions

Detailed inclusion and exclusion criteria for each subgroup are summarized in **Supplementary Table 2**. Of the final study cohort, patients who were newly diagnosed during the study period were distinguished from patients who received their first diagnosis prior to the study period according to claims data. Patients who fulfilled the criteria for the exacerbation and crisis event subgroups were drawn

Abbreviations: AChE, acetylcholinesterase; COVID-19, coronavirus disease 2019; CPI-U, Consumer Price Index for All Urban Consumers; CPT, Current Procedural Terminology; ED, emergency department; GERD, gastroesophageal reflux disease; gMG, generalized MG; HCRU, healthcare resource utilization; ICD, International Classification of Diseases; ICU, intensive care unit; IDV®, Integrated Dataverse®; IVIg, intravenous immunoglobulin; LOS, length of stay; LRP4, low-density lipoprotein receptor-related protein 4; MG, myasthenia gravis; MG-ADL, MG activities of daily living; MGFA, Myasthenia Gravis Foundation of America; MuSK, muscle-specific kinase; NDC, National Drug Code; NMJ, neuromuscular junction; NSIST, non-steroidal immunosuppressive treatment; oMG, ocular MG; PIS, post-intervention status; PLEX, plasma exchange; SCIg, subcutaneous immunoglobulin; SD, standard deviation; US, United States.

from the final study cohort regardless of the time of their first diagnosis. The crisis event subgroup was defined by the presence of one or more intubation claims with an associated inpatient stay and ICU (intensive care unit) admission. The exacerbation event subgroup was defined only by the presence of MG exacerbation ICD codes G70.01 (ICD-10) or 358.01 (ICD-9) with a concomitant absence of intubation claims. Of note, reasons for the exacerbation claim and the accuracy of coding practices was indiscernible in the claims dataset (limitations are further addressed in the Discussion).

Time Period Selection

Each patient was associated with a gMG index date defined as the first occurrence of a diagnostic claim for MG filed by a non-ophthalmologic specialist between January 1, 2017 and December 31, 2018. Patients were considered newly diagnosed (ND) if the index date was their first diagnosis of MG in the available data, and previously diagnosed (PD) if their first diagnosis of MG in the available data occurred before the index date; the analysis period continued for 12 months following the index dates.

For the exacerbation event subgroup, the exacerbation index date was defined as the date of first acute exacerbation claim filed between January 1, 2017 and December 31, 2018; the analysis period continued for 12 months following the exacerbation index date.

For the crisis event subgroup, the crisis index date was defined as the date of first intubation claim filed between January 1, 2017 and December 31, 2018. The analysis period for the crisis event subgroup spanned across three time periods: pre-crisis, during the crisis, and post-crisis. The pre-crisis time period was defined as up to 3 years preceding the crisis index date (with intervals of 1 year). The crisis event start date was defined as the date of intubation (crisis index date), and the end date was defined as the last date of a continuous inpatient stay. The post-crisis time period was defined as 12 months following the crisis index date; thus, post-crisis time periods include the crisis event duration.

For each time period, continuous quarterly activity (defined as $\geq$1 claim filed per quarter) was assessed for missing data; patients were included in the cost analysis if they had at least 1 claim activity of any type (MG or non-MG) during each quarter within the time period of interest. December 31, 2018 was chosen as the end date for patient selection to allow a follow-up period of 12 months from diagnosis that ended before the beginning of the COVID-19 (coronavirus disease 2019) pandemic.

Study Measures

Baseline Demographics

Baseline demographics at the index date were analyzed for the final study cohort and subgroups. Parameters notated in the claims data included age, gender, comorbidities, and health insurance plan type at the index date. Mean $\pm$ standard deviation (SD) and median age were derived from patients' year of birth. Health insurance and plan type were defined as commercial, Medicare, Medicaid, or other. The five most frequently occurring comorbidities per ICD-10 diagnostic codes (excluding gMG-related diagnostic codes) within patients' claims were assessed.

Healthcare Resource Utilization

HCRU for medical services was evaluated using the place of service and procedure codes listed for each claim in the dataset. Visits were broadly classified into hospitalizations, outpatient services (defined as hospital outpatient and clinic visits), emergency department (ED) visits, office visits, and other services. Hospitalizations were defined as the continuous stay of a patient in a hospital/inpatient setting (identified on basis of place of service in the data), and mean hospitalizations per patient were calculated using distinct instances per patient for continuous inpatient stays. Outpatient, office, and other visits were determined using the unique number of corresponding claims and calculating mean visits per patient for each category. Mean ED visits per patient were identified using claims with procedure code descriptions for ED, critical care, hospital observation, and emergency service settings.

Treatment Pattern Analysis

Claims including treatment with intravenous immunoglobulin (IVIg) or subcutaneous immunoglobulin (SCIg), rituximab, eculizumab, plasma exchange (PLEX), acetylcholinesterase (AChE) inhibitors, non-steroidal immunosuppressive treatments (NSISTs), or corticosteroids were considered gMG-related treatments and included in treatment pattern analyses. NSISTs included azathioprine, cyclophosphamide, cyclosporine, methotrexate, mycophenolate, and tacrolimus.

Cost Analysis

Estimated paid amounts were derived from charged amounts for medical procedures provided in the IDV® dataset. Mean and total costs were evaluated for a 12-month period at the patient level from the payer perspective, and subset analyses were performed by separating total costs into medical service costs and pharmacy costs as detailed in the **Supplementary Methods**.

Direct costs for gMG treatments were estimated based on costs incurred for the following gMG-relevant therapies: standard-of-care therapies (AChE inhibitors, NSISTs, and corticosteroids) and add-on therapies (IVIg or SCIg, rituximab, eculizumab, or PLEX).

For the ND, PD, and exacerbation event subgroups, mean, total, medical, and pharmacy costs were estimated across the outpatient, inpatient, clinic, office, and other/unknown settings 12 months after the index date. For the crisis event subgroup, mean, total, medical, and pharmacy costs were analyzed annually for up to 3 years before the crisis event index date, during the crisis event, and 12 months following the crisis index date. All costs were standardized to 2018 US dollars using the CPI-U (Consumer Price Index for All Urban Consumers) provided by the US Bureau of Labor Statistics (https://data.bls.gov/timeseries/CUUR0000SAM?output_view=data). Further details on cost estimation methods are available in the **Supplementary Methods**.

TABLE 1 | Baseline patient demographic and clinical characteristics at index date.

Baseline characteristics	Total (*N* = 41,940)	ND (*n* = 12,822)	PD (*n* = 29,118)	Exacerbation event (*n* = 4,355)	Crisis event (*n* = 206)
Age, years					
Mean (SD)	64.78 (13.43)	65.46 (12.89)	64.48 (13.66)	65.57 (12.89)	63.70 (14.89)
Median (IQR)	69 (58–77)	69 (59–77)	69 (57–77)	69 (59–77)	68 (56–76)
Gender, n (%)*					
Male	20,116 (47.96)	6,325 (49.33)	13,791 (47.37)	2,047 (47.00)	95 (46.12)
Female	21,823 (52.04)	6,496 (51.66)	15,327 (52.63)	2,308 (53.00)	111 (53.88)
Unknown	1 (0)	1 (0.01)	0 (0)	0 (0)	0 (0)
Mean age by gender, years (SD)*					
Male	68.07 (10.82)	68.20 (10.37)	68.00 (11.02)	68.62 (10.07)	68.58 (9.71)
Female	61.75 (14.83)	62.79 (14.45)	61.31 (14.97)	62.87 (14.43)	59.52 (17.13)
Health insurance type, n (%)†					
Commercial	33,912 (80.86)	10,419 (81.26)	23,493 (80.68)	3,641 (83.60)	188 (91.26)
Medicare	23,975 (57.16)	7,365 (57.44)	16,610 (57.04)	2,645 (60.70)	133 (64.56)
Medicaid	4,361 (10.40)	1,240 (9.67)	3,121 (10.72)	489 (11.20)	39 (18.93)
Other‡	3,797 (9.05)	1,208 (9.42)	2,589 (8.89)	456 (10.50)	31 (15.05)
Pharmacy insurance type, n (%)†					
Commercial	18,823 (44.88)	5,791 (45.16)	13,032 (44.76)	1,953 (44.80)	90 (43.69)
Medicare	20,282 (48.36)	6,301 (49.14)	13,981 (48.01)	2,230 (51.20)	107 (51.94)
Medicaid	5,103 (12.17)	1,527 (11.91)	3,576 (12.28)	570 (13.10)	36 (17.48)
Other‡	12,909 (30.78)	4,263 (33.25)	8,646 (29.69)	1,563 (35.90)	82 (39.81)
Top five comorbidities (ICD-10 code), n (%)§					
Essential hypertension (I10)	22,928 (54.67)	7,242 (56.48)	15,686 (53.87)	2,590 (59.47)	173 (83.98)
Hyperlipidemia, unspecified (E78.5)	12,415 (29.60)	4,006 (31.24)	8,409 (28.88)	1,416 (32.51)	91 (44.17)
Type 2 DM without complications (E11.9)	10,287 (24.53)	3,176 (24.77)	7,111 (24.42)	1,275 (29.28)	97 (47.09)
GERD without esophagitis (K21.9)	8,829 (21.05)	2,828 (22.06)	6,001 (20.61)	1,041 (23.90)	79 (38.35)
Hypothyroidism, unspecified (E03.9)	7,829 (18.67)	2,482 (19.36)	5,347 (18.36)	933 (21.42)	42 (20.39)

DM, diabetes mellitus; GERD, gastroesophageal reflux disease; ICD, International Classification of Diseases; IQR, interquartile range; ND, newly diagnosed; PD, previously diagnosed; SD, standard deviation.

**Claims data denote gender, as opposed to sex. †Percentages may exceed 100% as some patients were covered under more than one plan type. ‡Other includes cash, government, pharmacy benefit managers, and unknown. §Percentages may exceed 100% due to a subset of patients having multiple comorbidities.*

Statistical Analysis

For baseline patient demographic and clinical characteristics in the overall cohort and patient subgroups, continuous variables were summarized using mean and SD, while binary and categorical variables, such as gender, insurance type, comorbidities, patients with ≥1 filed claim, and treatment patterns were described using frequencies and percentages. HCRU and costs were calculated as standardized mean (total HCRU or costs divided by number of patients in the cohort).

For comparisons, two sample *t*-tests were used to assess statistically significant differences in mean age between male and female patients. HCRU related to percentage of patients with ≥1 filed claim and treatment patterns in ND and PD patient subgroups were compared using Chi-squared tests. As data were not normally distributed, HCRU related to hospital visits and length of stay (LOS) in ND and PD subgroups were compared using Wilcoxon rank sum tests. For comparisons between time periods within the crisis event subgroup, McNemar tests and Wilcoxon signed-rank tests were used to account for dependency of data within the same individuals.

Analyses were conducted using R 4.0.4 (R Foundation for Statistical Computing, Auckland, New Zealand), and statistical significance was assessed at the 0.05 level. Detailed statistical analysis results are reported in the **Supplementary Tables 2, 3, 5, 8**, and **9**.

RESULTS

Study Cohort

Of patients with claims including MG diagnostic codes between January 1, 2014 and December 31, 2019 identified in the dataset, 66,119 patients who fulfilled the study inclusion criteria and had at least 1 claim filed between January 1, 2017 and December 31, 2018 were further screened. From this cohort, 1,560 patients whose claims were associated with only ophthalmologic specialists were excluded from further analysis. Of the 41,490 total patients with continuous quarterly claims activity included in the final study cohort, 12,822 patients were identified as ND and 29,118 patients were identified as PD with gMG prior to the study period. Regardless of the time of first diagnosis, 4,355 patients fulfilled criteria for the exacerbation

TABLE 2 | Standardized healthcare resource utilization in ND, PD, and exacerbation event subgroups over 12 months.

Healthcare resource unit	ND (n = 12,822)	PD (n = 29,118)	Exacerbation event (n = 4,355)
Hospitalizations			
Patients with ≥1 filed claim, n (%)	3,032 (23.64)	5,472 (18.79)	1,569 (36.02)
12-month hospitalizations, standardized mean	0.46	0.34	0.72
LOS (days), standardized mean	1.39	0.99	2.04
ED visits			
Patients with ≥1 filed claim, n (%)	4,023 (31.37)	8,415 (28.90)	1,661 (38.41)
12-month ED visits, standardized mean	1	0.88	1.24
Outpatient visits			
Patients with ≥1 filed claim, n (%)	9,465 (73.81)	20,707 (71.11)	3,250 (74.62)
12-month outpatient visits, standardized mean	7.33	6.92	8.76
Office visits			
Patients with ≥1 filed claim, n (%)	10,751 (83.84)	24,110 (82.80)	3,576 (82.11)
12-month office visits, standardized mean	8.76	7.98	9.49
Other visits*			
Patients with ≥1 filed claim, n (%)	6,224 (48.54)	14,114 (48.47)	2,516 (57.77)
12-month office visits, standardized mean	4.25	4.57	6.59

ED, emergency department; LOS, length of stay; ND, newly diagnosed; PD, previously diagnosed.
**Other visits included care provided in settings that did not fall within other defined categories such as independent laboratories, home health agencies, and hospices.*

event subgroup and 206 patients for the crisis event subgroup (**Supplementary Figure 1**).

Baseline Demographics

Table 1 summarizes baseline demographics of the final study cohort and subgroups at the index date. Mean (SD) age of the total study cohort was 64.78 (13.43) years (median [interquartile range]: 69 [58–77] years). Mean age of female patients (61.75 years) was significantly lower than that of male patients (68.07 years; $p < 0.001$), suggesting female patients were diagnosed at a younger age.

Commercial plans and Medicare were the most common health insurance types observed among the total study cohort, while pharmacy insurance was spread between commercial plans, Medicare, and other sources. Medicaid was less utilized among the study cohort across both health and pharmacy insurance plans compared with Medicare and commercial plans.

The most frequent comorbidity identified was essential hypertension followed by hyperlipidemia, type 2 diabetes mellitus, gastroesophageal reflux disease (GERD), and hypothyroidism (**Table 1**; **Supplementary Table 3**). While the prevalence of hypertension (12), diabetes (13), and GERD (14) within the study cohort were largely consistent with age-matched national reporting, the occurrence of hyperlipidemia (15) and hypothyroidism (16) trended higher in the study cohort compared with data based on a general population. Notably, all 5 of the most frequent comorbidities were overrepresented among patients in the crisis event subgroup compared with the total study cohort.

Healthcare Resource Utilization

Compared with PD patients, ND patients had significantly higher HCRU with greater mean hospitalizations, longer mean LOS, and increased mean ED, outpatient, and office visits over 12 months ($p < 0.001$) (**Table 2**). A large proportion of ND patients with hospitalizations was observed in the first quarter of the year following the index date, which then stabilized over the remaining quarters (**Supplementary Table 4**).

HCRU was further increased for patients who experienced exacerbation events, with markedly higher values observed across hospitalizations, LOS, ED, outpatient, and office visits compared with the larger ND or PD cohorts ($p < 0.001$) (**Table 2**). For patients who experienced crisis events, HCRU during the 36–25 months and 24–13 months leading up to the crisis index date was comparable to or lower than that observed in the larger ND or PD cohorts; however, a dramatic increase in HCRU was observed during the 12 months immediately preceding the crisis event ($p < 0.001$) (**Table 3**). During this time period, more than a 2-fold increase in mean hospitalizations, LOS, and ED visits was observed compared with the two preceding years. As expected, mean LOS and ED visits markedly increased during crisis events. In the 12-month period following the crisis index date (which included the crisis event duration), mean hospitalizations, LOS, and ED visits increased 2- to 3-fold further when compared with the 12-month period preceding the crisis event ($p < 0.001$).

Treatment Pattern Analysis

Treatment patterns between ND and PD patients were similar, with the most frequently used medications being AChE inhibitors, corticosteroids, and NSISTs (**Table 4**). While patients

TABLE 3 | Standardized healthcare resource utilization in the crisis event subgroup.

Healthcare resource unit	Pre-crisis ($n = 206$)			Crisis event* ($n = 206$)	Post-crisis† ($n = 206$)
	36–25 months	24–13 months	12–0 months		0–12 months
Hospitalizations					
Patients with ≥1 filed claim, n (%)	47 (22.81)	52 (25.24)	125 (60.68)	206 (100)	206 (100)
12-month hospitalizations, standardized mean	0.46	0.50	1.35	1.00	2.78
LOS (days), standardized mean	1.27	1.47	3.60	15.38	10.14
ED visits					
Patients with ≥1 filed claim, n (%)	62 (30.09)	74 (35.92)	133 (64.56)	205 (99.51)	206 (100)
12-month ED visits, standardized mean	1.24	1.34	3.01	5.65	8.78
Outpatient visits					
Patients with ≥1 filed claim, n (%)	141 (68.44)	142 (68.93)	169 (82.03)	104§ (50.48)	184 (89.32)
12-month outpatient visits, standardized mean	7.00	7.43	11.34	1.26	16.32
Office visits					
Patients with ≥1 filed claim, n (%)	156 (75.72)	150 (72.81)	171 (83.00)	12 (5.83)	169 (82.03)
12-month office visits, standardized mean	6.31	6.40	9.31	0.10	10.00
Other visits‡					
Patients with ≥1 filed claim, n (%)	101 (49.02)	101 (49.02)	133 (64.56)	66 (32.03)	172 (83.49)
12-month office visits, standardized mean	4.02	4.60	6.47	0.66	15.33

ED, emergency department; LOS, length of stay.

**Crisis event HCRU was evaluated during hospital stay. †Post-crisis period included the crisis duration. ‡Other visits included care provided in settings that did not fall within other defined categories such as independent laboratories, home health agencies, and hospices. §Since visits were calculated based on respective claims, outpatient/office claims present in the data appear proportionately lower during crisis duration.*

TABLE 4 | Patient distribution within each drug class for ND, PD, and exacerbation event subgroups.

Therapeutic class, n (%)	ND ($n = 12,822$)	PD ($n = 29,118$)	Exacerbation event ($n = 4,355$)
IVIg + SCIg	1,016 (7.9)	2,387 (8.1)	755 (17.3)
Rituximab	99 (0.7)	348 (1.1)	66 (1.5)
Eculizumab	89 (0.6)	58 (0.1)	53 (1.2)
PLEX	354 (2.7)	695 (2.3)	286 (6.5)
AChE inhibitors	6,546 (51.1)	13,394 (45.9)	2,391 (54.9)
NSISTs*	3,053 (23.8)	8,222 (28.2)	1,477 (33.9)
Corticosteroids	5,986 (46.6)	12,293 (42.2)	2,344 (53.8)

AChE, acetylcholinesterase; IVIg, intravenous immunoglobulin; ND, newly diagnosed; NSIST, non-steroidal immunosuppressive treatment; PD, previously diagnosed; PLEX, plasma exchange; SCIg, subcutaneous immunoglobulin.

**NSISTs included azathioprine, cyclophosphamide, cyclosporine, methotrexate, mycophenolate, and tacrolimus.*

who experienced exacerbation events used similar standard-of-care therapies, more than double the proportion of patients in the exacerbation event subgroup reported use of rescue or add-on therapies including IVIg or SCIg (17.3%), PLEX (6.5%), and eculizumab (1.2%) compared with the ND and PD cohorts.

Treatment patterns during pre-crisis, crisis, and post-crisis periods were analyzed for patients in the crisis event subgroup (**Table 5**). Consistent with the HCRU increases observed, the proportion of patients receiving AChE inhibitors, corticosteroids, NSISTs, and PLEX significantly increased ($p < 0.01$) in the 12-month period immediately before the crisis event compared with the 2 preceding years, and were maintained or further increased during the 12 months following the crisis index date. Though eculizumab was not used by any patient in this subgroup during the 3 years preceding the crisis event, 2.4% of patients were prescribed eculizumab during the 12 months following the crisis index date. IVIg and SCIg use increased over the 3 years leading to the crisis event (4.4% to 7.8% to 8.7%) with a significant increase observed between the 12 months before and after the crisis index date (14.5%; $p < 0.05$).

Cost Analysis

Here, we report standardized mean payer-relevant costs; actual average costs per patient at an individual level are reported in **Supplementary Tables 5–8**.

ND, PD, and Exacerbation Event Subgroups

ND patients incurred higher mean all-cause costs compared with PD patients ($26,419.20, ND; $24,941.47, PD) (**Table 6**) (median costs: $7,300.27, ND; $6,681.28, PD; $p < 0.001$). Mean direct costs for gMG treatments were similarly higher for ND patients ($9,890.37) compared with PD patients ($9,186.47). As expected, patients in the exacerbation event subgroup incurred higher all-cause costs ($43,734.15) and direct costs for gMG treatments ($21,550.02) compared with the overall ND and PD cohorts.

For drug costs (**Table 7**), IVIg and SCIg costs represented 52.8% of total direct costs for gMG treatments for ND patients ($5,223.70 of $9,890.37) and 73.4% for PD patients ($6,743.17 of $9,186.47) despite only a small proportion of patients (7.9% of

TABLE 5 | Patient distribution within each drug class for the crisis event subgroup.

Therapeutic class, n (%)	Pre-crisis (n = 206)			Crisis event* (n = 206)	Post-crisis (n = 206)
	36–25 months	24–13 months	12–0 months		0–12 months
IVIg + SCIg	9 (4.4)	16 (7.8)	18 (8.7)	2 (1.0)	30 (14.5)
Rituximab	2 (1.0)	4 (1.9)	2 (1.0)	0 (0)	8 (3.9)
Eculizumab	0 (0)	0 (0)	0 (0)	0 (0)	5 (2.4)
PLEX	9 (4.4)	5 (2.4)	13 (6.3)	37 (17.9)	53 (25.7)
AChE inhibitors	67 (32.5)	73 (35.4)	111 (53.8)	19 (9.2)	117 (56.7)
NSISTs†	43 (20.9)	39 (18.9)	53 (25.7)	6 (2.9)	82 (39.8)
Corticosteroids	77 (37.4)	88 (42.7)	110 (53.4)	30 (14.6)	120 (58.3)

*AChE, acetylcholinesterase; IVIg, intravenous immunoglobulin; NSIST, non-steroidal immunosuppressive treatment; PLEX, plasma exchange; SCIg, subcutaneous immunoglobulin. *Most costs and drug utilization information during a crisis event were captured from inpatient claims. These inpatient costs are not directly attributable to gMG-specific therapies; hence, overall utilization numbers are underreported (nearly to 45%). †NSISTs included azathioprine, cyclophosphamide, cyclosporine, methotrexate, mycophenolate, and tacrolimus.*

TABLE 6 | Standardized mean 12-month payer-relevant medical and pharmacy service costs per patient in ND, PD, and exacerbation event subgroups.

Type of costs (USD)	ND (n = 12,822)	PD (n = 29,118)	Exacerbation event (n = 4,355)
Mx estimated paid amount			
All-cause costs	21,785.29	20,062.10	37,724.34
Direct costs for gMG treatments*	8,627.10	7,579.34	19,320.15
Unspecified gMG costs	7,676.81	7,788.92	12,123.51
Rx paid amount			
All-cause costs	4,633.91	4,879.36	6,009.82
Direct costs for gMG treatments*	1,263.27	1,607.14	2,229.87
Unspecified gMG costs	NA	NA	NA
Total paid amount			
All-cause costs	26,419.20	24,941.47	43,734.15
Direct costs for gMG treatments*	9,890.37	9,186.47	21,550.02
Unspecified gMG costs	7,676.81	7,788.92	12,123.51

gMG, generalized myasthenia gravis; Mx, medical services; NA, not applicable; ND, newly diagnosed; PD, previously diagnosed; Rx, pharmacy; USD, United States dollar.
**Direct costs for gMG treatments were calculated based on therapies relevant to gMG only. These were defined as intravenous immunoglobulin (IVIg) or subcutaneous immunoglobulin (SCIg), rituximab, eculizumab, plasma exchange (PLEX), acetylcholinesterase (AChE) inhibitors, non-steroidal immunosuppressive treatments (NSISTs), or corticosteroids.*

TABLE 7 | Standardized mean 12-month payer-relevant drug costs per patient in ND, PD, and exacerbation event subgroups.

Type of costs (USD)	ND (n = 12,822)	PD (n = 29,118)	Exacerbation event (n = 4,355)
Mx estimated paid amount			
IVIg or SCIg	4,954.15	6,299.06	12,757.53
Rituximab	312.59	514.66	894.45
Eculizumab	3,203.47	535.38	5,263.24
PLEX	150.78	220.60	396.59
AChE inhibitors	2.23	0.43	0.55
NSISTs*	2.45	7.57	3.39
Corticosteroids	1.44	1.63	4.42
Rx paid amount			
IVIg or SCIg	269.55	444.11	723.43
Rituximab	7.02	10.90	6.24
Eculizumab	133.99	83.77	361.49
PLEX	-	0.02	-
AChE inhibitors	570.00	700.03	749.95
NSISTs*	246.73	337.65	347.79
Corticosteroids	35.97	30.66	40.98
Total paid amount			
IVIg or SCIg	5,223.70	6,743.17	13,480.95
Rituximab	319.61	525.55	900.68
Eculizumab	3,337.46	619.16	5,624.73
PLEX	150.78	220.62	396.59
AChE inhibitors	572.23	700.47	750.50
NSISTs*	249.18	345.22	351.18
Corticosteroids	37.41	32.29	45.40

AChE, acetylcholinesterase; IVIg, intravenous immunoglobulin; Mx, medical services; ND, newly diagnosed; NSIST, non-steroidal immunosuppressive treatment; PD, previously diagnosed; PLEX, plasma exchange; Rx, pharmacy; SCIg, subcutaneous immunoglobulin; USD, United States dollar.
**NSISTs included azathioprine, cyclophosphamide, cyclosporine, methotrexate, mycophenolate, and tacrolimus.*

ND and 8.1% of PD) receiving IVIg or SCIg treatment (**Table 4**). IVIg and SCIg costs also comprised the highest proportion of drug costs for the exacerbation event subgroup despite only 17.3% of patients in the subgroup being prescribed IVIg or SCIg. Eculizumab incurred the second highest costs among drug classes for both the ND patient cohort and the exacerbation event subgroup, but not for the PD patient cohort.

Crisis Event Subgroup

For the crisis event subgroup, all-cause costs were significantly higher during the 12 months immediately preceding a crisis event ($49,236.68) compared with 24–13 months ($24,354.37) prior to the crisis event ($p < 0.001$) (**Table 8**). All-cause costs further increased more than 3-fold in the 12 months following the crisis index date ($173,956.99 [period included the crisis event duration]; $p < 0.001$). Direct costs for gMG treatments varied

TABLE 8 | Standardized mean 12-month payer-relevant medical and pharmacy service costs per patient in the crisis event subgroup.

Type of costs (USD)	Pre-crisis ($n = 206$)			Crisis event* ($n = 206$)	Post-crisis† ($n = 206$)
	36–25 months	24–13 months	12–0 months		0–12 months
Mx estimated paid amount					
All-cause costs	15,217.29	18,710.30	44,356.68	92,586.90	168,766.58
Direct costs for gMG treatments‡	2,604.48	6,013.05	5,141.30	353.69	23,569.42
Unspecified gMG costs	7,686.42	7,876.30	29,470.02	86,088.68	127,362.95
Rx paid amount					
All-cause costs	5,617.30	5,644.07	4,880.00	91.13	5,190.41
Direct costs for gMG treatments‡	2,717.65	2,554.05	1,908.58	21.15	1,505.87
Unspecified gMG costs	NA	NA	NA	NA	NA
Total paid amount					
All-cause costs	20,834.59	24,354.37	49,236.68	92,678.02	173,956.99
Direct costs for gMG treatments‡	5,322.13	8,567.10	7,049.88	374.84	25,075.29
Unspecified gMG costs	7,686.42	7,876.30	29,470.02	86,088.68	127,362.95

gMG, generalized myasthenia gravis; Mx, medical services; NA, not applicable; Rx, pharmacy; USD, United States dollar.

**Crisis event costs were evaluated during hospital stay. As hospital expenses were coded under diagnosis-related groups (DRGs), costs may show as bundled in claims data, limiting distinct identification of individual cost items during crisis hospitalization episodes. †Post-crisis period included the crisis duration. ‡Direct costs for gMG treatments were calculated based on therapies relevant to gMG only. These were defined as intravenous immunoglobulin (IVIg) or subcutaneous immunoglobulin (SCIg), rituximab, eculizumab, plasma exchange (PLEX), acetylcholinesterase (AChE) inhibitors, non-steroidal immunosuppressive treatments (NSISTs), or corticosteroids.*

across the time periods but were highest during the 12 months following the crisis index date. Unspecified gMG costs also rose significantly during the 12 months preceding the crisis event and continued to increase in the 12 months following the crisis index date ($p < 0.001$). Notably, unspecified gMG costs comprised a higher proportion of all-cause costs at most time points for the crisis event subgroup compared with ND, PD, or exacerbation event subgroups.

Similar to the ND, PD, and exacerbation event subgroups, the majority of drug costs for patients in the crisis event subgroup were attributed to IVIg or SCIg during all time periods analyzed, with a significant and marked increase in cost observed between the 12 months preceding crisis ($4,499.59) and the post-crisis period, which included the crisis duration ($12,488.30; $p < 0.001$) (**Table 9**). During the post-crisis period, eculizumab costs comprised the second highest proportion of drug costs despite being prescribed to only 2.4% of patients in this subgroup (**Table 5**). Costs incurred for all gMG-related treatment classes examined in this study were higher in the post-crisis time period compared with 36–25 months prior to a crisis event.

DISCUSSION

The present analysis of HCRU and costs associated with gMG in the US was informed through a robust algorithm validated by clinical experts to capture patients living with gMG within national claims data from Symphony Health's IDV® dataset. Although gMG has been associated with a substantial clinical and economic burden, few studies have highlighted the magnitude of this burden in the US (11, 17–23) despite having the highest direct medical cost of MG out of 8 countries with available data (10). A need for additional and updated studies—particularly focusing on patients' clinical characteristics—was reported in a recent systematic literature review (10). To address these knowledge gaps, in addition to evaluating HCRU, treatment patterns, and costs in patients with gMG who were ND, PD, and who experienced exacerbation events, we expanded our analysis for patients who experienced myasthenic crisis events to include 3 years leading up to the crisis event, during the crisis event, and 1 year following the crisis event.

Though values are not directly comparable due to differences in databases used, time periods analyzed, and cost estimation methodologies, mean per-patient annual direct medical costs for our larger study population ($26,419.20 for ND; $29,941.47 for PD) were in a consistent range with a previous US-based burden of illness report for MG ($28,780) (10, 11). Other past analyses using claims data from Symphony Health's IDV® dataset may aid in further contextualizing our findings in gMG. Mean 1-year per-patient all-cause costs from IDV® in 10,140 patients with epilepsy without tuberculosis sclerosis complex was reported to be $56,397 (24), while total cost over 5 years was reported at $47,464 (average of $9492.80 per year) for 15,599 patients with rheumatoid arthritis who used oral methotrexate only (25). For 7043 patients with chronic obstructive pulmonary disease, 1-year all-cause costs from IDV® were reported at $19,690.40 (26). Though direct comparisons cannot be made due to non-trivial differences in cost estimation algorithms (i.e., charged vs. paid amounts), it is apparent that costs for gMG lie in the higher range within these relatively age-matched chronic conditions, with exacerbations and crisis events causing costs to rise even further. Of note, previous reporting that the cost of MG management comprised 78% of total direct medical costs when compared with a matched non-MG control group suggests that the majority of healthcare expenses observed in our study can be attributed to gMG management (17).

TABLE 9 | Standardized mean 12-month payer-relevant drug costs per patient in the crisis event subgroup.

Type of costs (USD)	Pre-crisis (n = 206)			Crisis event* (n = 206)	Post-crisis† (n = 206)
	36–25 months	24–13 months	12–0 months		0–12 months
Mx estimated paid amount					
IVIg or SCIg	1,721.95	4,912.84	3,808.33	206.23	12,488.30
Rituximab	260.30	416.47	130.15	-	1,688.72
Eculizumab	-	-	-	-	6,948.66
PLEX	604.88	681.58	1,200.74	147.41	2,411.94
AChE inhibitors	0.07	0.04	0.23	-	0.11
NSISTs‡	15.27	0.13	1.04	-	23.83
Corticosteroids	2.02	1.99	0.82	0.05	7.87
Rx paid amount					
IVIg or SCIg	1,972.99	1,441.13	691.26	-	-
Rituximab	-	137.44	198.95	-	-
Eculizumab	-	-	-	-	-
PLEX	-	-	-	-	-
AChE inhibitors	585.33	812.45	844.40	14.05	884.19
NSISTs‡	137.50	139.58	152.59	5.89	570.36
Corticosteroids	21.83	23.45	21.37	1.21	51.32
Total paid amount					
IVIg or SCIg	3,694.94	6,353.96	4,499.59	206.23	12,488.30
Rituximab	260.30	553.91	329.10	-	1,688.72
Eculizumab	-	-	-	-	6,948.66
PLEX	604.88	681.58	1,200.74	147.41	2,411.94
AChE inhibitors	585.40	812.49	844.63	14.05	884.30
NSISTs‡	152.77	139.71	153.63	5.89	594.19
Corticosteroids	23.85	25.44	22.19	1.26	59.18

AChE, acetylcholinesterase; IVIg, intravenous immunoglobulin; Mx, medical services; NSIST, non-steroidal immunosuppressive treatment; PLEX, plasma exchange; Rx, pharmacy; SCIg, subcutaneous immunoglobulin; USD, United States dollar.

**Crisis event costs were evaluated during hospital stay. As hospital expenses were coded under diagnosis-related groups (DRGs), costs may show as bundled in claims data, limiting distinct identification of individual cost items during crisis hospitalization episodes. †Post-crisis period included the crisis duration. ‡NSISTs included azathioprine, cyclophosphamide, cyclosporine, methotrexate, mycophenolate, and tacrolimus.*

During interviews, clinical experts estimated the prevalence of gMG to be higher than that found in the literature and claimed that almost all patients require therapy at some point. They predicted that differential diagnoses, diagnostic delays, and the complexity of treatment decisions for ND gMG might lead to increased intervention and thus, increased healthcare costs when compared with PD gMG. Consistent with this, our results demonstrated higher 12-month HCRU (**Table 2**) and costs (**Table 6**) associated with ND gMG compared with PD gMG. Increased HCRU was impacted by greater mean hospitalizations, longer LOS, and greater mean ED visits, and increased costs were largely attributed to medical and pharmacy service costs. The pronounced proportion of ND patients with high HCRU associated with the time of new diagnosis suggests that previously undiagnosed patients may present with more severe symptoms or complications that require more intense clinical attention compared with PD patients.

A granular analysis of patients who experienced myasthenic crisis events yielded intriguing results. During the 36–25 months and 24–13 months preceding the crisis index date, HCRU and costs incurred were comparable to, or even lower than, corresponding results for ND and PD patient cohorts. However, a dramatic increase in HCRU and costs was observed during the year immediately preceding the crisis event, which further increased during the year following the crisis index date (which included crisis duration). Increased usage of rescue treatments including IVIg or SCIg, PLEX, eculizumab, and rituximab during these time periods contributed most to the increased costs (**Table 9**). These data suggest that myasthenic crisis events, which are key drivers of HCRU and cost for patients living with gMG, may be predictable and preventable; additional investigation around possible windows of opportunity and appropriate interventions are warranted.

Consistent with recommended treatment patterns in the literature (27), most patients in the study were observed to be undergoing treatment with AChE inhibitors, corticosteroids, and/or NSISTs (**Table 4**). Increased usage of monoclonal antibodies (eculizumab and rituximab), recommended for patients with severe symptoms with insufficient response to standard treatment (28), was observed in the exacerbation and

crisis event subgroups. Across all subgroups, costs for IVIg or SCIg treatment accounted for the greatest proportion of total drug costs (**Tables 7**, **9**), despite a small subset of patients utilizing them (**Tables 4**, **5**). Our results are consistent with a previous study reporting that IVIg accounted for 85% of MG-related pharmacy costs despite being used by 12% of patients with MG (11). Of note, the present study did not differentiate between rescue and maintenance IVIg treatment, and associated cost differences have not been investigated. Further, our study did not address the treatment-refractory patient subgroup as other recent studies have focused on examining their burden in detail (7–9, 29, 30).

A major limitation in the present study pertains to defining exacerbation events. Using solely ICD diagnostic codes as a proxy for gMG exacerbations can involve considerable caveats, as misuse or misclassification of diagnostic codes can occur; ICD codes for MG with or without acute exacerbation may be used interchangeably by mistake, and their usage may not totally reflect MG status. This is an inherent limitation associated with analysis of any administrative health claims data without a link to extensive medical records. Additionally, the occurrence of exacerbations can be subjective depending on the individual patient's history, rate of disease progression, and bulbar involvement, and etiologies associated with exacerbation claims were not available in the data. Considering these factors, it should be noted that this subgroup may not capture those with true exacerbations to the full extent. Nevertheless, we found that this subgroup, which included a considerable proportion of patients, had higher HCRU and costs compared with the ND and PD patient cohorts. Despite the limitations, higher usage of later-line treatments (e.g., IVIg, SCIg, and PLEX) suggests this distinct subgroup of patients had a clearly more severe disease profile requiring greater clinical intervention compared with the overall population of patients with gMG.

Some other limitations in our study should be noted. First, the mean age range of the study population is higher compared with some previous studies (11, 21). Though the number of late-onset MG diagnoses are rapidly increasing (18, 27, 28), this alone cannot account for the gap. We confirmed that while our initial cohort had a mean age more aligned to a previous insurance database study (11), the mean age increased as each of the following study inclusion criteria were applied: (1) having at least 2 MG claims at least 30 days apart, (2) adults aged $\geq$18 years, (3) index date between 2017 and 2018, and (4) continuous quarterly claims activity. While we recognize that these criteria led to the enrichment of older patients who may seek increased medical care while excluding a proportion of younger and healthier patients, they were critical to ensure a robust analysis and to maintain consistency with standard practice for longitudinal analyses of open claims data. Next, the present study could not account for key socioeconomic and demographic factors unavailable in the dataset (including race and ethnicity) that are additional underlying drivers of resource use and costs. Critical factors pertaining to disease severity, including MG activities of daily living (MG-ADL), the Myasthenia Gravis Foundation of America (MGFA) class, and MGFA post-intervention status (PIS) are also not available in claims data and must be investigated through other databases. However, some additional patient subgroup stratifications that are feasible using available data in claims (including age, gender, key comorbidities, and geographic location) are currently being analyzed in a separate ongoing follow-up study to address further knowledge gaps pertaining to the burden of gMG in various patient subpopulations.

Further, claims are subject to inconsistencies in diagnostic and procedural coding practices; although the inclusion and exclusion criteria sought to limit cases involving misdiagnoses (e.g., chronic fatigue syndrome or fibromyalgia), they did not account for patients with gMG who had not received a diagnosis within the time periods of interest. Any change in the patients' point-of-care location or benefits enrollment during a quarter with claims activity, as well as any services provided outside of the Symphony Health (Blue Bell, PA, USA) provider network, may have led to missed events. Next, though our analysis took multiple measures to exclude patients with oMG with added considerations for disease progression from oMG to gMG, separate diagnostic codes between oMG and gMG are necessary to establish further targeted insights. Additionally, the IDV® dataset was provider-based (rather than insurance-based) with records unavailable for patient eligibility. Medical expenditures were reported as charged amounts, which are different from actual paid amounts. To address these limitations in the dataset, expert guidance was incorporated into our unique cost estimation algorithms elaborated in **Supplementary Methods**. Lastly, our definition for direct costs for gMG treatments did not capture costs associated with HCRU, other medical services, or the management of comorbidities. Further comparison of patients with gMG against a claims-based non-gMG population will lend insights into the incremental costs and HCRU of gMG management relative to a general population.

In the present study of patients living with gMG, we observed that ND patients had a higher HCRU and cost burden compared with PD patients, which could be attributed to severe and/or progressed disease symptoms requiring robust treatment, or confounding symptoms leading to differential diagnoses, diagnostic delays, and the complexity of treatment decisions. Additional research is required to better understand the potential drivers of increased HCRU and costs of exacerbation and crisis events by delineating socioeconomic factors and intensity of interventions. Furthermore, real-world reimbursement data should be utilized to validate our novel algorithm. To lower costs and improve outcomes for patients with gMG, providers can monitor and identify risk factors for crisis events to achieve prevention or timely intervention. Importantly, there is a need to reduce IVIg and SCIg costs which account for a major portion of cost to payers, and preventive treatment options that could minimize the risk of crisis events should be made available to relieve the cost burden of patients with gMG in the US.

DATA AVAILABILITY STATEMENT

The data analyzed in this study was obtained from Symphony Health (https://symphonyhealth.prahs.com/), the following

licenses/restrictions apply: The data analyzed in this study are subject to restrictions due to a license agreement between Symphony Health (Blue Bell, PA, USA) and ZS Associates (Evanston, IL, USA). Requests to access these datasets should be directed to Symphony Health, https://symphonyhealth.prahs.com/contact/inquiry.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CA, AG, AW, and SB were involved in data analysis, statistical analysis, and drafting the manuscript. GP, YL, DG, and EB contributed to data interpretation and refining methods. All authors were involved in developing the concept and designing the study methodology, contributed to the article, and approved the submitted version.

FUNDING

This study was funded by argenx US Inc., Boston, MA, USA. The funder was involved in the study design, interpretation of aggregate results, manuscript reviews, and the decision to submit for publication. The funder did not have access to raw data obtained from Symphony Health, Blue Bell, PA, USA.

ACKNOWLEDGMENTS

The authors thank Dr. Pushpa Narayanaswami (Beth Israel Deaconess Medical Center, Boston, MA, USA), Dr. Gregory Sahagian (The Neurology Center of Southern California, Carlsbad, CA, USA), Dr. A. Gordon Smith (Virginia Commonwealth University, Richmond, VA, USA), and Dr. Gil Wolfe (University at Buffalo, Buffalo, NY, USA) for participating in expert interviews. David Anderson (Northwestern University Feinberg School of Medicine, Chicago, IL, USA) provided advisory and expert support for cost analyses using claims data. Sudhir Jadhav and Arpit Bhuwalka (ZS Associates, Pune, Maharashtra, India) performed the claims data analyses. Minxuan Huang, Ph.D. (ZS Associates, New York, NY, USA) performed the statistical analyses. Medical writing and editorial support was provided by Sahiba Randhawa, MPharm (ZS Associates, Gurgaon, Haryana, India); Mai Sato, Ph.D. (ZS Associates, New York, NY, USA); and Aparna Sharma, MPharm (ZS Associates, Gurgaon, Haryana, India) in accordance with the Good Publication Practice (GPP3) guidelines (http://www.ismpp.org/gpp3) and was funded by argenx US Inc., Boston, MA, USA. Part of the material in this manuscript was published in Value in Health, Volume 24, Supplement 1, S163. Phillips GA, et al. PND27 Cost-of-illness for adults with generalized myasthenia gravis in the US. Copyright Elsevier (2021).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.809999/full#supplementary-material

REFERENCES

1. Conti-Fine BM, Milani M, Kaminski HJ. Myasthenia gravis: past, present, and future. *J Clin Investig.* (2006) 116:2843–54. doi: 10.1172/JCI29894
2. Carr AS, Cardwell CR, McCarron PO, McConville J. A systematic review of population based epidemiological studies in myasthenia gravis. *BMC Neurol.* (2010) 10:46. doi: 10.1186/1471-2377-10-46
3. Hendricks TM, Bhatti MT, Hodge DO, Chen JJ. Incidence, epidemiology, and transformation of ocular myasthenia gravis: a population-based study. *Am J Ophthalmol.* (2019) 205:99–105. doi: 10.1016/j.ajo.2019.04.017
4. Myasthenia Gravis Foundation of America. *Clinical Overview of MG.* (2015). Available online at: https://myasthenia.org/Professionals/Clinical-Overview-of-MG (accessed July 2, 2021).
5. Jani-Acsadi A, Lisak RP. Myasthenic crisis: guidelines for prevention and treatment. *J Neurol Sci.* (2007) 261:127–33. doi: 10.1016/j.jns.2007.04.045
6. Thomas CE, Mayer SA, Gungor Y, Swarup R, Webster E, Chang I, et al. Myasthenic crisis: clinical features, mortality, complications, and risk factors for prolonged intubation. *Neurology.* (1997) 48:1253–60. doi: 10.1212/WNL.48.5.1253
7. Engel-Nitz NM, Boscoe A, Wolbeck R, Johnson J, Silvestri NJ. Burden of illness in patients with treatment refractory myasthenia gravis. *Muscle Nerve.* (2018) 58:99–105. doi: 10.1002/mus.26114
8. Murai H, Hasebe M, Murata T, Utsugisawa K. Clinical burden and healthcare resource utilization associated with myasthenia gravis: assessments from a Japanese claims database. *Clin Exp Neuroimmunol.* (2019) 10:61–8. doi: 10.1111/cen3.12495
9. Sharma A, Roy B, Khokhar B, Nowak R. Burden of disease in patients with refractory myasthenia gravis (P5.4-037). *Neurology*. (2019) 92:P5.4–037.
10. Landfeldt E, Pogoryelova O, Sejersen T, Zethraeus N, Breiner A, Lochmüller H. Economic costs of myasthenia gravis: a systematic review. *Pharmacoeconomics.* (2020) 38:715–28. doi: 10.1007/s40273-020-00912-8
11. Guptill JT, Marano A, Krueger A, Sanders DB. Cost analysis of myasthenia gravis from a large US insurance database. *Muscle Nerve.* (2011) 44:907–11. doi: 10.1002/mus.22212
12. Fryar CD, Ostchega Y, Hales CM, Zhang G, Kruszon-Moran D. Hypertension prevalence and control among adults: United States, 2015-2016. *NCHS Data Brief.* (2017) 1–8.
13. U.S. Department of Health and Human Services. *Centers for Disease Control and Prevention. National Diabetes Statistics Report.* (2020). Available online at: https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf (accessed November 24, 2021).
14. El-Serag HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut.* (2014) 63:871–80. doi: 10.1136/gutjnl-2012-304269
15. QuickStats: Prevalence of high total cholesterol among adults aged ≥20 years, by age group and sex — National Health and Nutrition Examination Survey, 2015–2018. *MMWR Morb Mortal Wkly Rep.* (2020) 69:690. doi: 10.15585/mmwr.mm6922a5
16. Aoki Y, Belin RM, Clickner R, Jeffries R, Phillips L, Mahaffey KR. Serum TSH and total T4 in the United States population and their association with participant characteristics: National Health and Nutrition

Examination Survey (NHANES 1999-2002). *Thyroid.* (2007) 17:1211–23. doi: 10.1089/thy.2006.0235
17. Guptill JT, Sharma BK, Marano A, Soucy A, Krueger A. Sanders DB. Estimated cost of treating myasthenia gravis in an insured US population. *Muscle Nerve.* (2012) 45:363–6. doi: 10.1002/mus.22327
18. Souayah N, Nasar A, Suri MF, Kirmani JF, Ezzeddine MA, Qureshi AI. Trends in outcomes and hospitalization charges among mechanically ventilated patients with myasthenia gravis in the United States. *Int J Biomed Sci.* (2009) 5:209–14. Available online at: http://www.ijbs.org/User/ContentFullText.aspx?VolumeNO=5&StartPage=209
19. Desai R, Abbas SA, Fong HK, Lodhi MU, Doshi R, Savani S, et al. Burden and impact of takotsubo syndrome in myasthenic crisis: a national inpatient perspective on the under-recognized but potentially fatal association. *Int J Cardiol.* (2020) 299:63–6. doi: 10.1016/j.ijcard.2019.09.054
20. Heatwole C, Johnson N, Holloway R, Noyes K. Plasma exchange versus intravenous immunoglobulin for myasthenia gravis crisis: an acute hospital cost comparison study. *J Clin Neuromuscul Dis.* (2011) 13:85–94. doi: 10.1097/CND.0b013e31822c34dd
21. Mandawat A, Kaminski HJ, Cutter G, Katirji B, Alshekhlee A. Comparative analysis of therapeutic options used for myasthenia gravis. *Ann Neurol.* (2010) 68:797–805. doi: 10.1002/ana.22139
22. Narla S, Silverberg JI. Associations of pemphigus or pemphigoid with autoimmune disorders in US adult inpatients. *J Am Acad Dermatol.* (2020) 82:586–95. doi: 10.1016/j.jaad.2019.07.029
23. Omorodion JO, Pines JM, Kaminski HJ. Inpatient cost analysis for treatment of myasthenia gravis. *Muscle Nerve.* (2017) 56:1114–8. doi: 10.1002/mus.25624
24. Betts KA, Stockl KM, Yin L, Hollenack K, Wang MJ, Yang X. Economic burden associated with tuberous sclerosis complex in patients with epilepsy. *Epilepsy Behav.* (2020) 112:107494. doi: 10.1016/j.yebeh.2020.107494
25. Lee J, Pelkey R, Gubitosa J, Henrick MF, Ganz ML. Comparing healthcare costs associated with oral and subcutaneous methotrexate or biologic therapy for rheumatoid arthritis in the United States. *Am Health Drug Benefits.* (2017) 10:42–9.
26. Ding B, Kallenbach L, Slipski L, Wilk A, O'Brien D, Guranlioglu D. Patient characteristics and healthcare resource utilization among patients with COPD new to LAMA/LABA fixed-dose combination treatment in US-based real-world practice. *Int J Chron Obstruct Pulmon Dis.* (2020) 15:775–86. doi: 10.2147/COPD.S238408
27. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol.* (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3
28. Gilhus NE, Tzartos S, Evoli A, Palace J, Burns TM, Verschuuren J. Myasthenia gravis. *Nat Rev Dis Primers.* (2019) 5:30. doi: 10.1038/s41572-019-0079-y
29. Boscoe AN, Xin H, L'Italien GJ, Harris LA, Cutter GR. Impact of refractory myasthenia gravis on health-related quality of life. *J Clin Neuromuscul Dis.* (2019) 20:173–81. doi: 10.1097/CND.0000000000000257
30. Harris L, Aban IB, Xin H, Cutter G. Employment in refractory myasthenia gravis: a Myasthenia Gravis Foundation of America registry analysis. *Muscle Nerve.* (2019) 60:700–6. doi: 10.1002/mus.26694

Conflict of Interest: GP, DG, and EB are employees of argenx US Inc., Boston, MA, United States. YL is a paid consultant for and receives grant support from argenx US Inc., Boston, MA, United States. CA, AG, AW, and SB are employees of ZS Associates New York, NY, United States; Princeton, NJ, United States; Bangalore, India and serve as paid consultants for argenx US Inc., Boston, MA, United States.

frontiers in Neurology

ORIGINAL RESEARCH
published: 04 February 2022
doi: 10.3389/fneur.2022.816243

Clinical Predictors of Relapse in a Cohort of Steroid-Treated Patients With Well-Controlled Myasthenia Gravis

*Shengyao Su, Lin Lei, Zhirong Fan, Shu Zhang, Qi Wen, Jingsi Wang, Yan Lu, Li Di, Min Wang, Hai Chen and Yuwei Da**

Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China

Objective: Despite the high efficiency of glucocorticoids (GCs), ∼18–34% patients with myasthenia gravis (MG) may experience relapses of the disease. Here, we aim to identify clinical factors related to relapses during steroid tapering or after withdrawal in MG patients who were well-managed on steroid monotherapy.

Methods: We conducted a retrospective study on 125 MG patients from the Xuanwu Hospital MG Trial Database. Patients were treated with corticosteroids and achieved minimal manifestation status (MMS) or better. Patients were divided into steroid reduction subset ($N = 74$) and steroid withdrawal subset ($N = 51$). Clinical characteristics and therapeutic data were compared between patients with disease relapse and those who maintained clinical remission at the last follow-ups. Cox proportional hazards regression models were used to identify risk factors of relapse in each subset.

Results: Thirty-seven (29.6%) patients experienced relapses during the follow-up periods. Relapse during the steroid reduction was significantly associated with drug reducing duration (HR = 0.81, 95%CI 0.74–0.89, $P < 0.001$). Risk of relapse was augmented if the drug reducing duration was < 11.5 months (HR 27.80, 95%CI 5.88–131.57, $P < 0.001$). Among patients who discontinued the steroids, those with onset symptoms of bulbar weakness (adjusted HR 3.59, 95%CI 1.19–10.81, $P = 0.023$) were more likely to experience relapse.

Conclusion: Our study demonstrated that patients could benefit from prolonged steroid-reducing duration to prevent disease relapse. Patients with bulbar weakness at disease onset should be proposed to take long-term steroids or other immunosuppressants.

Keywords: myasthenia gravis, relapse, steroid monotherapy, clinical predictor, steroid reduction, steroid withdrawal

OPEN ACCESS

Edited by:
Nils Erik Gilhus, University of Bergen, Norway

Reviewed by:
Michelangelo Maestri, University of Pisa, Italy
Deepak Menon, University Health Network (UHN), Canada

****Correspondence:***
Yuwei Da
dayuwei100@hotmail.com

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *16 November 2021*
Accepted: *05 January 2022*
Published: *04 February 2022*

Citation:
Su S, Lei L, Fan Z, Zhang S, Wen Q, Wang J, Lu Y, Di L, Wang M, Chen H and Da Y (2022) Clinical Predictors of Relapse in a Cohort of Steroid-Treated Patients With Well-Controlled Myasthenia Gravis. Front. Neurol. 13:816243. doi: 10.3389/fneur.2022.816243

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease with the presence of autoantibodies against the neuromuscular junction proteins. Treatments such as acetylcholinesterase inhibitors, immunotherapies, thymectomy, intravenous immunoglobulin (IVIG), and plasma exchange are used to realize the therapeutic target of full physical function and high quality

of life (1). Despite the fact that promising novel therapies are upcoming (2), the glucocorticoid (GC) is still the first choice of MG therapy on the basis of rapid onsets of effects, low costs, and high efficiency, which could lead to improvement in 80–95% patients (3–6). After the relief of symptoms, the corticosteroid dose is reduced or even discontinued to minimize the accompanying side effects of long-term use (3–6). However, ∼18–34% patients may experience subsequent exacerbations or disease relapses (5, 7, 8). Only 10–20% patients could discontinue immunotherapy completely and achieve complete stable remission (CSR) (4, 9, 10).

It has been demonstrated that the increased risk of relapse was correlative with drug withdrawal or rapid reduction of steroids when patients took corticosteroid as monotherapy in the 1990's (5, 6). Thereafter, the "slow and steady" tapering strategy was adopted in the clinical practice when the steroid was administrated solely (11). Even so, patients may still experience disease recurrence during the drug reduction. In some cases, relapses of MG occur in months to years after the discontinuation of prednisone (6). However, there are few studies concerning the clinical factors that are correlative with relapse during steroid tapering (5, 6, 12). Moreover, to our knowledge, risk factors of relapse after steroid withdrawal have not been investigated thoroughly.

Here, we present a retrospective cohort analysis of GC-treated MG patients from a single center in order to determine indicators of clinical relapse under steroid monotherapy.

MATERIALS AND METHODS

Patients and Ethical Statements

Medical records and follow up data of consecutive MG patients from the Xuanwu Hospital Capital Medical University Myasthenia Gravis Trial Database since April 2017 to July 2020 were retrospectively reviewed and analyzed. The study was approved by the Ethics Committee of Xuanwu Hospital (No. 2017084). All patients provided written informed consents.

The inclusion criteria included: (1) Patients were diagnosed with MG and over 16 years. The diagnosis of MG was based on fluctuating weakness symptoms along with supporting pharmacological, serologic, and electrophysiologic tests (13). (2) Patients were treated with GC for controlling disease and the maintenance therapy in the absence of other immunosuppressive agents, except for short-term IVIG during the acute exacerbations. Steroids were prescribed for at least 1 month before patients reached a stable status. The stable status was defined as patients having no symptoms of functional limitations from MG, meeting the criteria for minimal manifestation status (MMS) or better according to the MGFA postintervention status (MGFA-PIS) classification (14). (3) Patients were followed up prospectively after the enrollment for at least 12 months. We identified 154 potential patients who were receiving GC therapies. The exclusion criteria were as follows: (1) patients had incomplete medical records or less than one follow-up visit. (2) Patients took other immunosuppressants, except for short-term IVIG. Patients whose therapies switched to other immunosuppressive agents due to steroid-induced side effects during the follow-up period were excluded. (3) Patients experienced relapses before enrollments. (4) Patients achieved stable status at the last visits with no further follow-up information. Ultimately, 125 patients were enrolled (**Figure 1**).

Clinical Features and Evaluations

Clinical features were collected including sex, the age of onset, onset symptoms, symptoms at nadir, MGFA classification at the nadir, presence of autoantibodies, repetitive nerve stimulation tests (RNS) result, and presence of other autoimmune diseases. The presenting symptoms within the first month of disease onsets were collected as the onset symptoms. Mild disease was defined as MGFA II class at disease maximal worsening, and moderate to severe disease was defined as MGFA III to V classes. Radiographic examinations of the mediastinum were performed routinely, and 31 patients underwent thymectomies. Patients with thymoma (N=17) were pathologically diagnosed. MG–activities of daily living (ADL) scores were measured to quantify the disease severities. Follow-up assessments were scheduled every 3 months for the first year and then every 6 months. Assessments included clinical symptoms, ADL scores, prednisone doses, and the dates of achieving stable status. Once patients achieved MMS or better, they did not need to come for the return visits and telephone follow-ups would be performed. The follow-ups for all the enrolled patients were performed prospectively and completed by July 2021.

Treatment

All the enrolled patients were taking steroids as monotherapy. The short-term use of IVIG during the acute exacerbation was permitted. Whether patients were treated with high dose intravenous methylprednisolone (IVMP) pulses or not was also recorded. The induction therapy regimens were categorized as steroid increasing regimen, medium-dose regimen, and steroid-tapering regimen. After patient's marked improvements or reaching MMS or better, the maximal GC doses would be tapered to the minimal doses, if the conditions permitted. The tapering strategy comprised a 5 mg reduction monthly or slower until it reached 20 mg daily, 5 mg reduction every 2 to 4 months until 5 mg daily. A 5 mg dose per day of steroid would be discontinued in 3–6 months. Patients whose GC doses were reduced but not discontinued were grouped into steroid reduction subset (SR subset; $N = 74$), and patients who discontinued steroids were into steroid withdrawal subset (SW subset; $N = 51$).

The doses, start and end dates at the initiation, maximum and the final doses of oral corticosteroids were recorded. The final steroid dose was noted as the minimum dose before relapse or at the final visit. The intervals between the steroid initiation to the maximum doses, the steroid initiation to the stable status, and the drug reducing duration were calculated by month. The drug reducing duration (month) was defined as the interval from the end date of the maximum dose of oral steroids to the start date of the final dose. The accumulated dose of oral steroids before stable status was counted according to the medical record and periodic follow-up records. The average reduction speed (mg/month) was computed as the difference between the maximal dose and the final dose, divided by the drug reducing duration. Duration of

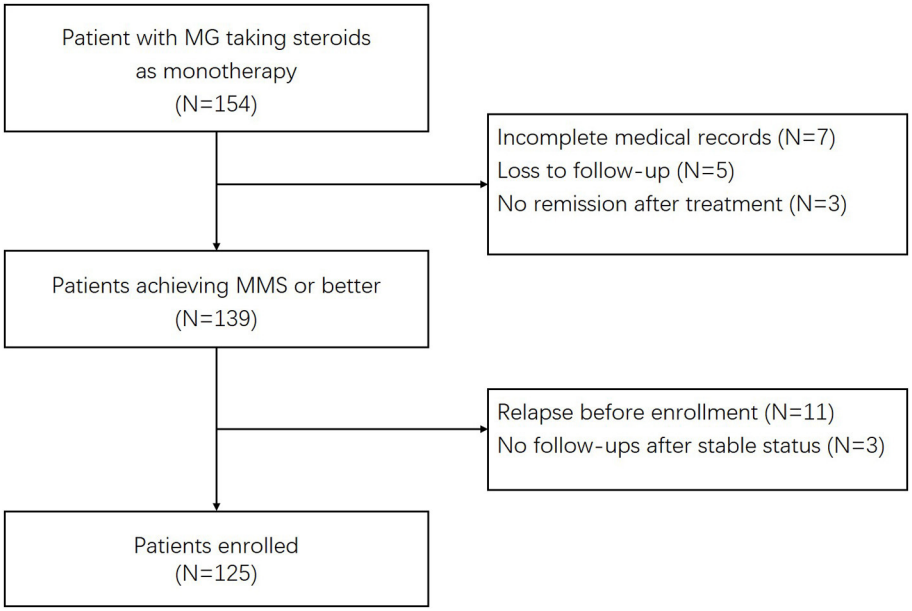


FIGURE 1 | Flowchart of the participants included in the current study.

the final dose was the interval from the start of the final dose to the last follow-up or relapse, whichever came first. In SW subset, the start date of the final dose was when the patient stopped the corticosteroid.

Relapse

The primary endpoint was the occurrence of disease relapse, which was defined as recurrence of MG symptoms or a substantial increase in MG medications after the patient achieving MMS or better status (14). If patients didn't pay return visits during disease exacerbations, symptoms, and ADL-scores of relapses would be inquired by telephone. In this case, increases of ADL scores were determined as disease relapse. Patients of each subset were divided into relapse group (R-MG) and non-relapse group (NR-MG). Clinical features and therapeutic data were compared between R-MG and NR-MG groups to find relevant factors of relapse.

Statistical Analysis

Continuous variables were presented as median and interquartile range (IQR), and categorical variables were presented as number and frequency. Clinical characteristics were compared between R-MG and NR-MG. Continuous data were analyzed using the Mann–Whitney U test. Categorical variables were analyzed using the chi-square or Fisher exact test, as appropriate. Candidate variables were included in Cox proportional hazards regression for calculating the multivariable hazard ratio (HR) if univariate *P*-values were < 0.10. Kaplan–Meier curves of relapse rates were plotted to illustrate the differences over time. Patients were censored at the last follow-up visits. The receiver operating characteristic (ROC) curve method was used to evaluate the best cut-off value of drug reducing duration in predicting relapse. Data were analyzed using SPSS (Version 22, IBM) and Prism (version7, GraphPad). A value of *P* lower than 0.05 was regarded as significant.

RESULTS

The Primary Endpoint and the Therapy Regimens of All the Enrolled Patients

A relapse rate of 29.6% (37/125) was observed in the current study, and the median time from stable status to relapse was 18 months (IQR 8.0–21.5, range 2.0–53.0). Basic clinical characteristics and therapeutic data of the 125 patients are shown in **Table 1**. Ninety-seven patients had pure ocular symptoms at onset, six patients had pure limb weakness at onset, and seven patients had pure bulbar symptoms. Fifteen patients presented with more than one symptom at onset. No patient presented with shortness of breath at onset. The induction therapy regimens varied and could be generally categorized as steroid-increasing regimen, medium-dose regimen, and steroid-tapering regimen. Fifty-eight patients (46.4%) took low initial doses (median 15.0 mg/day, IQR 15.0–16.3) and the dosages gradually increased to maximal doses (median 35.0 mg/day, IQR 30.0–50.0) until improvement was observed; 45 patients (36.0%) took medium doses (median 25.0 mg/day, IQR 17.5–30.0) as maintenance therapy; and 22 patients (17.6%) initiated high dose corticosteroid treatments (median 60.0 mg/day, IQR 50.0–60.0), after which the dosages were gradually tapered. Other than drug reduction or discontinuation, the reported causes of relapse included over exertion ($N = 4$), cold ($N = 3$), and pneumonia ($N = 1$). Thirty-one patients experienced one MG relapse and six patients had two relapses during the follow-up periods.

TABLE 1 | Clinical characteristics and therapeutic data of enrolled patients on steroid monotherapy.

	Total (*N* = 125)	Steroid reduction subset (*N* = 74)			Steroid withdrawal subset (*N* = 51)		
		Relapse group (*N* = 19)	Non-relapse group (*N* = 55)	*P*-value[a]	Relapse group (*N* = 18)	Non-relapse group (*N* = 33)	*P*-value[b]
Age at onset (years)	48.0 (IQR 35.0–59.0)	52.0 (IQR 42.0–63.0)	49.0 (IQR 34.0–57.5)	0.369	48.5 (IQR 36.5–59.0)	43.0 (IQR 28.5–62.0)	0.413
Sex (male)	73 (58.4%)	12 (63.2%)	29 (52.7%)	0.303	12 (66.7%)	20 (60.6%)	0.669
Symptoms at onset							
Ocular	111 (88.8%)	19 (100.0%)	47 (85.5%)	0.081	15 (83.3%)	30 (90.9%)	0.354
Limb	13 (10.4%)	1 (5.3%)	8 (14.5%)	0.267	0 (0.0%)	4 (12.1%)	0.164
Bulbar	17 (13.6%)	0 (0.0%)	9 (16.4%)	0.058	6 (33.3%)	2 (6.1%)	0.017
Symptoms at nadir							
Ocular	116 (92.8%)	19 (100.0%)	52 (94.5%)	0.405	15 (83.3%)	30 (90.9%)	0.354
Limb	34 (27.2%)	2 (10.5%)	18 (32.7%)	0.060	5 (27.8%)	9 (27.3%)	0.608
Bulbar	46 (36.8%)	6 (31.6%)	25 (45.5%)	0.291	8 (44.4%)	7 (21.2%)	0.082
Respiratory	8 (6.4%)	1 (5.3%)	5 (9.1%)	0.513	1 (5.6%)	1 (3.0%)	0.657
Disease severity at nadir				0.861			0.353
OMG (MGFA I)	64 (51.2%)	9 (47.4%)	24 (43.6%)		9 (50.0%)	22 (66.7%)	
Mild (MGFA II)	52 (41.6%)	9 (47.4%)	26 (47.3%)		7 (38.9%)	10 (30.3%)	
Moderate to severe (MGFA III-V)	9 (7.2)	1 (5.3%)	5 (9.1%)		2 (11.1%)	1 (3.0%)	
Autoimmune antibodies				0.323			0.097
AChR	90 (72.0%)	12 (63.2%)	42 (76.4%)		16 (88.9%)	20 (60.6%)	
MuSK	8 (6.4%)	3 (15.8%)	3 (5.5%)		0 (0.0%)	2 (6.1%)	
DN	27 (21.6%)	4 (21.2%)	10 (18.2%)		2 (11.1%)	11 (33.3%)	
RNS result	65 (52.0%)	9 (47.4%)	29 (52.7%)	0.445	9 (50.0%)	18 (54.4%)	0.756
Thymoma	17 (13.6%)	2 (10.5%)	10 (18.2%)	0.362	4 (22.2%)	1 (3.0%)	0.047
Thymectomy	33 (26.4%)	3 (15.8%)	17 (30.9%)	0.201	5 (27.8%)	6 (18.2%)	0.325
Presence of other autoimmune disease	6 (4.8%)	1 (5.3%)	4 (7.3%)	0.618	0 (0.0%)	1 (3.0%)	0.647
ADL score at nadir	4.0 (IQR 3.0–6.0)	4.0 (IQR 3.0–7.0)	4.0 (IQR 3.0–6.0)	0.844	5.0 (IQR 4.0–7.8)	4.0 (IQR 3.0–6.0)	0.254
Age at start of GC (years)	49.0 (IQR 35.5–59.5)	54.0 (IQR 44.0–63.0)	50.0 (IQR 36.0–59.0)	0.284	48.5 (IQR 36.5–59.0)	45.0 (IQR 31.5–62.0)	0.436
Disease course before immunotherapy (month)	5.0 (IQR 2.0–14.0)	6.0 (IQR 3.0–43.0)	5.0 (IQR 3.0–12.0)	0.434	4.0 (IQR 1.8–12.0)	2.0 (IQR 1.0–9.0)	0.445
Initial oral GC dose (mg/day)	20.0 (IQR 15.0–30.0)	15.0 (IQR 10.0–20.0)	20.0 (IQR 15.0–30.0)	0.242	20.0 (IQR 15.0–36.3)	20.0 (IQR 15.0–40.0)	0.772
Maximal oral GC dose (mg/day)	35.0 (IQR 25.0–50.0)	25.0 (IQR 20.0–50.0)	35.0 (IQR 25.0–50.0)	0.197	40.0 (IQR 28.8–60.0)	30.0 (IQR 20.0–47.5)	0.090
Final oral GC dose (mg/day)	5.0 (IQR 0.0–6.9)	5.0 (IQR 5.0–10.0)	5.0 (IQR 5.0–10.0)	0.445	0	0	-
Duration of the final dose (month)	6.0 (IQR 2.0–13.0)	3.0 (IQR 1.0–6.0)	5.0 (IQR 0.0–9.0)	0.396	4.0 (IQR 2.0–9.5)	17.0 (IQR 11.5–26.5)	<0.001
GC dose regimen of induction therapy				0.272			0.058
Steroid tapering regimen	22 (17.6%)	2 (10.5%)	8 (14.5%)		4 (22.2%)	8 (24.2%)	

(Continued)

TABLE 1 | Continued

	Total (*N* = 125)	Steroid reduction subset (*N* = 74)			Steroid withdrawal subset (*N* = 51)		
		Relapse group (*N* = 19)	Non-relapse group (*N* = 55)	*P*-value[a]	Relapse group (*N* = 18)	Non-relapse group (*N* = 33)	*P*-value[b]
Medium dose regimen	45 (36.0%)	9 (47.4%)	15 (27.3%)		4 (22.2%)	17 (51.5%)	
Steroid increasing regimen	58 (46.4%)	8 (42.1%)	32 (58.2%)		10 (55.6%)	8 (24.2%)	
Duration from GC initiation to stable condition (month)	3.0 (IQR 2.0–5.0)	2.0 (IQR 1.0–4.0)	3.0 (IQR 2.0–5.0)	0.090	3.0 (IQR 2.8–6.5)	2.0 (IQR 1.0–3.5)	0.024
Accumulated GC doses before stable status (mg)	2390.0 (IQR 1415.0–4495.0)	2055.0 (IQR 1350.0–3600.0)	3000.0 (IQR 1825.0–4950.0)	0.066	2727.5 (IQR 2185.0–6493.8)	1425.0 (IQR 900.0–2730.0)	0.009
Drug reducing duration (month)	13.0 (IQR 7.5–19.5)	8.0 (IQR 5.0–11.0)	15.0 (IQR 12.0–23.0)	<0.001	13.5 (IQR 3.0–20.75)	9.0 (IQR 3.5–16.0)	0.417
Average reduction speed(mg/month)	2.5 (IQR 1.4–5.0)	2.5 (IQR 1.4–6.3)	1.7 (IQR 1.0–3.5)	0.086	3.2 (2.2–13.8)	3.8 (1.9–8.8)	0.890
IVIG before remission	14 (11.2%)	1 (5.3%)	7 (12.7%)	0.337	3 (16.7%)	3 (9.1%)	0.354
IVMP before remission	7 (5.6%)	0 (0.0%)	4 (7.3%)	0.296	2 (11.1%)	1 (3.0%)	0.282

[a]*P-value was compared between R-MG and NR-MG in the steroid reduction subset;* [b]*P-value was compared between R-MG and NR-MG in the steroid withdrawal subset; MGFA, Myasthenia Gravis Foundation of America classification; AChR, acetylcholine receptor; MuSK, muscle specific kinase; DN, double negative; ADL, Activities of Daily Living; GC, glucocorticoid; IVIG, intravenous immunoglobulin; IVMP, intravenous methylprednisolone; IQR, interquartile range.*

Factors Correlative With Relapse During Steroid Reduction (SR)

Seventy-four patients were included in the SR subset, and 19 of them (25.7%) relapsed (**Table 1**). The median time from stable status to relapse was 16 months (IQR 7.0–19.0, range 2.0–31.0). Nine patients (12.2%) relapsed within the first year after achieving stable status and 17 patients (23.0%) relapsed within the first 2 years.

Among clinical characteristics and therapeutic data between R-MG and NR-MG groups, the drug reducing duration was the only factor associated with relapse (R-MG median 8.0 months, IQR 5.0–11.0 vs. NR-MG median 15.0 months, IQR 12.0–23.0, $P < 0.001$). There were no statistical differences in sex, age at onset, onset symptom, disease severity at nadir, MG-ADL score at nadir, MG autoantibody, RNS result, thymoma, thymectomy or presence of other autoimmune diseases. Ages at the initiation of corticosteroid, disease courses before treatment, initial steroid doses, maximal doses, intervals from steroid initiation to stable condition and the number of patients taking IVIG or IVMP therapies were comparable between R-MG and NR-MG groups. The median steroid dose of patients at relapse was 5 mg/day (IQR 5.0–10.0), which was similar to that of NR-MG patients at last follow-ups (median 5 mg/day, IQR 5.0–10.0, $P = 0.445$). Patients in the R-MG group had shorter durations from steroid initiation to stable status (median 2.0 months, IQR 1.0–4.0 vs. NR-MG median 3.0 months, IQR 2.0-5.0; $P = 0.090$), lower accumulated doses of oral steroids before stable status (median 2055.0 mg, IQR 1350.0–3600.0 vs. NR-MG median 3000.0 mg, IQR 1825.0–4950.0; $P = 0.066$), and faster average reduction speeds of steroids (median 2.5 mg/month, IQR 1.4–6.3 vs. NR-MG median 1.7 mg/month, IQR 1.0–3.5; $P = 0.086$), which, however, didn't reach the statistical significance. In using the Cox proportional hazards model to identify the prognostic covariates associated with relapse during tapering steroid doses, day 0 was defined as the date when the patient achieved stable status. Only shorter drug reducing duration was identified as a significant predictor of relapse (HR = 0.81, 95%CI 0.74–0.89, $P < 0.001$; **Table 2**). Using the ROC curve, the best cut-off value of drug reducing duration (month) was 11.5 (sensitivity 74.5%, specificity 78.9%, area under the curve 0.779). Risk of relapse was augmented if the drug reducing duration was < 11.5 months (HR 27.80, 95%CI 5.88–131.57, $P < 0.001$, **Figure 2**).

Factors Correlative With Relapse After Steroid Withdrawal (SW)

Fifty-one patients were included in the SW subset (**Table 1**). The occurrence of CSR was observed in 25/125 (20.0%) enrolled MG patients. Eighteen (35.3%) patients experienced relapses after drug discontinuation, and 15 of them relapsed within 12 months. The median time from stable status to relapse was 20 months (IQR 8.0–23.5, range 3.0–53.0). The median time from steroid withdrawal to relapse was 4 months (IQR 2.0–9.5, range 1.0–40.0). In patients who discontinued the steroids, relapse was associated with the onset symptom of bulbar weakness ($P = 0.017$; odds ratio: 7.75, 95%CI 1.37–43.87), thymoma ($P = 0.047$; odds ratio: 9.14, 95%CI 0.94–89.35), duration from steroid

TABLE 2 | Associations of clinical and therapeutic variables with relapse of patients on steroid reduction therapy ($N = 74$).

Clinical and therapeutic variables	HR	95% CI	*P*-value
Univariate			
Drug reducing duration (month)	0.81	0.74–0.89	<0.001
Drug reducing duration < 11.5 months	27.80	5.88–131.57	<0.001
Ocular weakness at onset	24.22	0.05–12422.17	0.317
Bulbar weakness at onset	0.04	0.00–17.62	0.301
Limb weakness at nadir	3.37	0.78–14.58	0.105
Duration from GC initiation to stable condition (month)	0.90	0.71–1.12	0.339
Accumulated GC doses before stable status (mg)	1.00	1.00–1.00	0.570
Average reduction speed(mg/month)	1.02	0.97–1.07	0.422
Thymectomy	1.87	0.54–6.44	0.321

Variables were included in multivariate analyses if P < 0.10 in univariate analyses; GC, glucocorticoid; HR, hazard ratio.

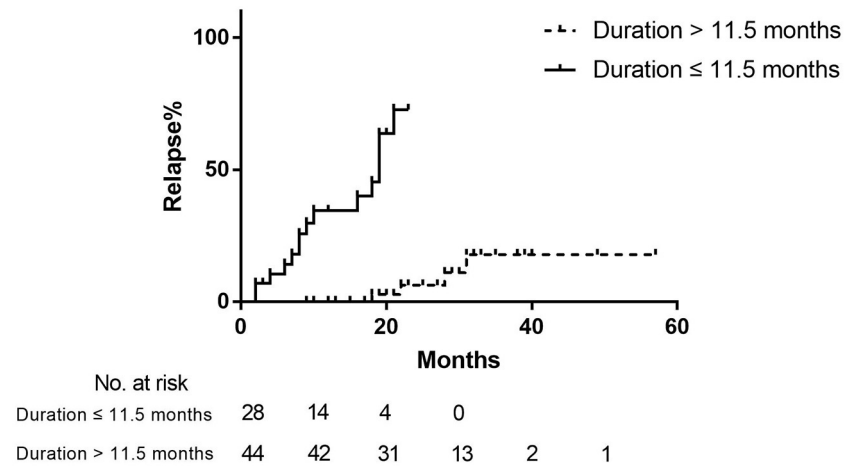


FIGURE 2 | Kaplan–Meier curve of the relapse rate in SR patients with different steroid-reducing durations. Day 0 was defined as the date when patients achieved stable status.

initiation to stable status (R-MG median 3.0 months, IQR 2.8–6.5 vs. NR-MG median 2.0 months, IQR 1.0–3.5; $P = 0.024$) and accumulated steroid doses before stable status (R-MG median 2727.5 mg, IQR 2185.0–6493.8 vs. NR-MG median 1425.0, IQR 900.0–2730.0; P=0.009). In Cox proportional hazards regression analysis, day 0 was defined as the steroid discontinuation date. Bulbar weakness at onset was identified to have a significant association with relapses after steroid discontinuation (adjusted HR 3.59, 95%CI 1.19–10.81, $P = 0.023$; **Table 3**, **Figure 3**). The median time from steroid discontinuation to relapse for patients with bulbar onset was 4.0 months (IQR 2.0–21.0, range 0.0–27.0).

DISCUSSION

We demonstrated a relapse rate of 29.6% in a cohort of well-managed MG patients taking GC as monotherapy and 20.0% of enrolled patients achieved CSR by the end of the study, which was consistent with previous studies (5, 7–10). Analysis implied that shortened GC reducing duration was a significant predictor for relapse during steroid tapering in the well-controlled MG patients. The bulbar symptom at disease onset was independently associated with relapse after the discontinuation of GC.

Among patients in SR subset of the current study, 25.7% patients relapsed. Most relapses (17/19, 89.5%) happened within the first 2 years after achieving stable status. R-MG group had significantly shorter drug reducing duration than NR-MG, and there were numerical trends of less duration from steroid initiation to stable status, lower accumulated steroid doses before stable status and higher steroid-reducing speed in R-MG group, which were approaching significance, whereas no statistical difference was found in clinical characteristics. Moreover, shortness of steroid-reducing duration was identified to be associated with increased risk of relapse during steroid tapering by the Cox proportional hazards model. These results implied that relapses during steroid reduction were more relevant to inadequate treatments. It was validated that once generalized MG patients attained the MMS, depending on the efficacy of azathioprine, rapid tapering of prednisone was associated with good outcomes and well tolerated without destabilizing MG (15). However, when the steroid was administered in absence of other

TABLE 3 | Associations of clinical and therapeutic variables with relapse after steroid discontinuation ($N = 51$).

Clinical and therapeutic variables	HR	95% CI	*P* value
Univariate			
Bulbar weakness at onset	4.34	1.59–11.85	0.004
Bulbar weakness at nadir	2.36	0.93–6.00	0.071
Thymoma	3.06	0.99–9.41	0.051
Thymectomy	0.62	0.22–1.76	0.369
Autoimmune antibodies			
AChR	Ref	-	0.309
MuSK	0.00	-	0.985
DN	0.32	0.07–1.38	0.125
GC dose regimen of induction therapy			
Steroid tapering regimen	Ref.	-	0.197
Medium dose regimen	0.40	0.10–1.62	0.200
Steroid increasing regimen	1.15	0.36–3.73	0.813
Maximal oral GC dose (mg/day)	1.02	0.99–1.04	0.209
Duration from GC initiation to stable status (month)	1.05	0.99–1.12	0.131
Accumulated GC doses before stable status (mg)	1.00	1.00–1.00	0.132
Multivariate			
Bulbar weakness at onset	3.59	1.19–10.81	0.023
Thymoma	1.82	0.53–6.28	0.342
Multivariate			
Bulbar weakness at nadir	2.20	0.86–5.67	0.101
Thymoma	2.77	0.89–8.64	0.079

Variables were included in multivariate analyses if $P < 0.10$ in univariate analyses; AChR, acetylcholine receptor; MuSK, muscle specific kinase; DN, double negative; GC, glucocorticoid; HR, hazard ratio.

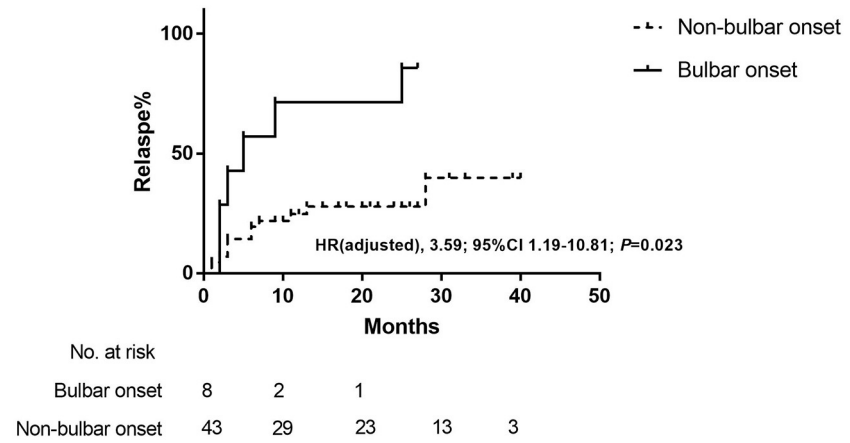


FIGURE 3 | Kaplan–Meier curve of the relapse rate after steroid discontinuation in patients with bulbar onset and non-bulbar onset. Day 0 was defined as the steroid discontinuation date. HR indicated hazard ratio.

immunosuppressants, it was well acknowledged that rapid dose-reduction could result in a recurrence of weakness (5, 11, 12). In line with these data, we found that risk of relapse increased by 26-fold when the steroid-reducing durations of the patients were < 11.5 months. The findings led us to conclude that the steroid-reducing duration of at least 1 year might be in favor of preventing disease relapse. It was close to statistically significant that a relatively high average reduction speed was observed in R-MG groups. The result was in accordance with less drug reducing duration in R-MG and might reach significance when expanding the sample size. The final doses before relapses in our study were similar to the minimum doses in the NR-MG groups, which was 5 mg/day (IQR 5.0–10.0). Low-dose medication could preserve well management of MG (11, 16). The side effects resulting from long-term use of steroids were dose-dependent, which could be minimized and acceptable by administration of

dosages no more than 5 mg (17, 18). In Japanese guidelines for MG, MM with oral prednisolone (PSL) of 5 mg/day or below was recommended as the therapeutic goal (19), which was more reachable than CSR and with equivalent satisfaction of patients (20). Since only shorter drug reducing duration was identified as a significant predictor of relapse, we presumed that with steroid-reducing duration longer than 1 year, patients might maintain asymptomatic on oral steroids of 5 mg per day.

Among patients who stopped GC therapies, 35.3% patients relapsed and most relapses (15/18, 83.3%) happened within the first year after GC discontinuation. To our knowledge, this is the first report concerning prognostic factors of relapse after steroid discontinuation. In the present study, bulbar weakness at onset was identified as a predictor of relapse in patients who discontinued steroids. Manifestations of bulbar symptoms included dysarthria, dysphagia, and dysphonia (21, 22), which might be the initial and solitary presentation in 15–27% MG patients (23, 24). The bulbar symptom was reported to be one of risk factors of the postsurgery myasthenia crisis (25). The relationship between the onset phenotype involving bulbar muscles and elevated relapse risk had not yet been published yet. Presence of thymoma and severe forms of MG were identified as risk factors of relapse in a cohort of steroid-treated MG patients (6). However, it would be more reasonable if they had performed a multivariate analysis and considered the confounding factors. It was demonstrated that patients with thymoma were generally in serious conditions (26). In agreement with pervious study, we observed a significantly higher proportion of patients with thymoma in R-MG, whereas it did not achieve statistical significance in the Cox regression analysis. However, it should be taken into account that our study was limited by the small sample size. In the present study, durations of oral steroid and accumulated GC doses before the stable status were significantly higher in R-MG of the SW subset, indicating that severe diseases might be related to relapses. Nevertheless, there were no differences in ADL scores or disease severity at nadir. This can be explained by the fact that patients in the current study were generally with mild to moderate diseases, since the median ADL score at maximal worsening of the cohort was 4 points. Patients with severe forms might take combining non-steroidal immunotherapies (8, 11) and were excluded from the current study. Taken together, bulbar weakness at onset could be indicative of relapse after GC discontinuation and patients might require long-term use of steroids or other immunosuppressants.

Our findings could not be ascribed to the confounding effects of MG antibodies or thymectomy, as the autoantibodies and thymectomized patients did not statistically significantly differ between R-MG group and NR-MG group. Our results coincided with the previous report that no significant correlation was found between thymectomy and relapse (7). Even though thymectomy was validated in controlling diseases and sparing prednisone doses in non-thymomatous generalized MG patients (27), relapse remained a major concern after discontinuing pharmacotherapy in thymectomized patients (28). This might be attributed to the fact that the disease relevant lymphocytes shuttled from thymus into circulation and resided in secondary sites of chronic pathogenic antibody production (29, 30). Thus, precautions should be taken against disease relapses when thymectomized patients become symptom-free and discontinue the immunotherapies. MuSK-MG was demonstrated to be associated with a higher risk of relapse (8). However, when comparing our results to the previous study, it must be pointed out that the majority of MuSK-MG patients in our cohort were ascribed to other immunotherapies and were excluded from the current study.

The main limitation of our work was the retrospective design and the limited sample size from a single center. Besides, follow-ups were completed mainly by telephones after patients achieved stable status. Therefore, the maintenance of stable conditions was based on the self-reports of patients, instead of careful physical examinations. Because of the retrospective design, patients who switched to other immunotherapies due to steroid-induced side effects during the follow-up periods were excluded from the study. Therefore, steroid maintenance therapies were well tolerated in the current cohort and the side effects of steroid were not measured and compared between groups.

In conclusion, despite the satisfactory effects of corticosteroids, about 30% well-managed patients with MG might experience disease relapses. Our study emphasized the significance in prolonged steroid-reducing durations of at least 1 year before reaching maintenance doses to prevent relapses. Moreover, laryngological manifestations at the onset of a disease might predict a high risk of relapse after discontinuance of GC, and these patients should be proposed to take long-term steroids or other immunosuppressants.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Xuanwu Hospital, Capital Medical University, China (No. 2017084). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SS contributed to this work in study design, collecting data, statistical analysis, drafting, and revising the manuscript. LL, ZF, SZ, QW, JW, YL, LD, MW, and HC contributed with the enrollment of patients and acquisition of data. YD contributed with study concept and design, drafting, revising the manuscript, and interpretation of the data. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Clinical Cohort Study of Myasthenia Gravis, National Key R&D Program of China, Precision Medicine Project (No. 2017YFC0907700), and the National Natural Science Foundation of China (No. 62171299).

REFERENCES

1. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S, et al. Myasthenia gravis - autoantibody characteristics and their implications for therapy. *Nat Rev Neurol.* (2016) 12:259–68. doi: 10.1038/nrneurol.2016.44
2. Menon D, Barnett C, Bril V. Novel treatments in myasthenia gravis. *Front Neurol.* (2020) 11:538. doi: 10.3389/fneur.2020.00538
3. Bedlack RS, Sanders DB. Steroid treatment for myasthenia gravis: steroids have an important role. *Muscle Nerve.* (2002) 25:117–21. doi: 10.1002/mus.1221
4. Sanders DB, Evoli A. Immunosuppressive therapies in myasthenia gravis. *Autoimmunity.* (2010) 43:428–35. doi: 10.3109/08916930903518107
5. Pascuzzi RM, Coslett HB, Johns TR. Long-term corticosteroid treatment of myasthenia gravis: report of 116 patients. *Ann Neurol.* (1984) 15:291–8. doi: 10.1002/ana.410150316
6. Evoli A, Batocchi AP, Palmisani MT, Lo MM, Tonali P. Long-term results of corticosteroid therapy in patients with myasthenia gravis. *Eur Neurol.* (1992) 32:37–43. doi: 10.1159/000116785
7. Wang L, Zhang Y, He M. Clinical predictors for the prognosis of myasthenia gravis. *BMC Neurol.* (2017) 17:77. doi: 10.1186/s12883-017-0857-7
8. Zhang C, Bu B, Yang H, Wang L, Liu W, Duan R-S, et al. Immunotherapy choice and maintenance for generalized myasthenia gravis in China. *Cns Neurosci Ther.* (2020) 26:1241–54. doi: 10.1111/cns.13468
9. Morren J, Li Y. Maintenance immunosuppression in myasthenia gravis, an update. *J Neurol Sci.* (2020) 410:116648. doi: 10.1016/j.jns.2019.116648
10. Baggi F, Andreetta F, Maggi L, Confalonieri P, Morandi L, Salerno F, et al. Complete stable remission and autoantibody specificity in myasthenia gravis. *Neurology.* (2013) 80:188–95. doi: 10.1212/WNL.0b013e31827b907b
11. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. *Neurology.* (2016) 87:419–25. doi: 10.1212/WNL.0000000000002790
12. Scherpbier HJ, Oosterhuis HJ. Factors influencing the relapse risk at steroid dose reduction in myasthenia gravis. *Clin Neurol Neurosurg.* (1987) 89:145–50. doi: 10.1016/S0303-8467(87)80046-X
13. Gilhus NE, Tzartos S, Evoli A, Palace J, Burns TM, Verschuuren JJGM, et al. Myasthenia gravis. *Nat Rev Dis Primers.* (2019) 5:30. doi: 10.1038/s41572-019-0079-y
14. Jaretzki AR, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. task force of the medical scientific advisory board of the myasthenia gravis foundation of America. *Ann Thorac Surg.* (2000) 70:327–34. doi: 10.1016/S0003-4975(00)01595-2
15. Sharshar T, Porcher R, Demeret S, Tranchant C, Gueguen A, Eymard B, et al. Comparison of corticosteroid tapering regimens in myasthenia gravis: a randomized clinical trial. *JAMA Neurol.* (2021) 78:426–33. doi: 10.1001/jamaneurol.2020.5407
16. Salins S, Teter B, Kavak K, Wolfe GI, Silvestri NJ. Low-dose medication and long-term outcome in myasthenia gravis. *J Clin Neuromuscul Dis.* (2016) 18:61–6. doi: 10.1097/CND.0000000000000122
17. Huscher D, Thiele K, Gromnica-Ihle E, Hein G, Demary W, Dreher R, et al. Dose-related patterns of glucocorticoid-induced side effects. *Ann Rheum Dis.* (2009) 68:1119–24. doi: 10.1136/ard.2008.092163
18. Strehl C, Bijlsma JW, de Wit M, Boers M, Caeyers N, Cutolo M, et al. Defining conditions where long-term glucocorticoid treatment has an acceptably low level of harm to facilitate implementation of existing recommendations: viewpoints from an EULAR task force. *Ann Rheum Dis.* (2016) 75:952–7. doi: 10.1136/annrheumdis-2015-208916
19. Murai H. Japanese clinical guidelines for myasthenia gravis: Putting into practice. *Clin Exp Neuroimmunol.* (2015) 6:21–31. doi: 10.1111/cen3.12180
20. Masuda M, Utsugisawa K, Suzuki S, Nagane Y, Kabasawa C, Suzuki Y, et al. The MG-QOL15 Japanese version: validation and associations with clinical factors. *Muscle Nerve.* (2012) 46:166–73. doi: 10.1002/mus.23398
21. Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. *Muscle Nerve.* (2008) 37:141–9. doi: 10.1002/mus.20950
22. Montero-Odasso M. Dysphonia as first symptom of late-onset myasthenia gravis. *J Gen Intern Med.* (2006) 21:C4–6. doi: 10.1111/j.1525-1497.2006.00343.x
23. Basiri K, Ansari B, Okhovat AA. Life-threatening misdiagnosis of bulbar onset myasthenia gravis as a motor neuron disease: how much can one rely on exaggerated deep tendon reflexes. *Adv Biomed Res.* (2015) 4:58. doi: 10.4103/2277-9175.151874
24. Mao VH, Abaza M, Spiegel JR, Mandel S, Hawkshaw M, Heuer RJ, et al. Laryngeal myasthenia gravis: report of 40 cases. *J Voice.* (2001) 15:122–30. doi: 10.1016/S0892-1997(01)00012-1
25. Geng Y, Zhang H, Wang Y. Risk factors of myasthenia crisis after thymectomy among myasthenia gravis patients: a meta-analysis. *Medicine.* (2020) 99:e18622. doi: 10.1097/MD.0000000000018622
26. Evoli A, Minisci C, Di Schino C, Marsili F, Punzi C, Batocchi AP, et al. Thymoma in patients with MG: characteristics and long-term outcome. *Neurology.* (2002) 59:1844–50. doi: 10.1212/01.WNL.0000032502.89361.0C
27. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo H-C, Marx A, et al. Long-term effect of thymectomy plus prednisone versus prednisone alone in patients with non-thymomatous myasthenia gravis: 2-year extension of the MGTX randomised trial. *Lancet Neurol.* (2019) 18:259–68. doi: 10.1016/S1474-4422(18)30392-2
28. Yu S, Li F, Chen B, Lin J, Yang M, Fu X, et al. Eight-year follow-up of patients with myasthenia gravis after thymectomy. *Acta Neurol Scand.* (2015) 131:94–101. doi: 10.1111/ane.12289
29. Ingelfinger F, Krishnarajah S, Kramer M, Utz SG, Galli E, Lutz M, et al. Single-cell profiling of myasthenia gravis identifies a pathogenic T cell signature. *Acta Neuropathol.* (2021). doi: 10.1007/s00401-021-02299-y
30. Jiang R, Fichtner ML, Hoehn KB, Pham MC, O'Connor KC. Single-cell repertoire tracing identifies rituximab-resistant B cells during myasthenia gravis relapses. *JCI Insight.* (2020) 5:e136471. doi: 10.1172/jci.insight.136471

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

ORIGINAL RESEARCH
published: 08 February 2022
doi: 10.3389/fneur.2022.820205

Long-Term Improvement in a Chinese Cohort of Glucocorticoid-Resistant Childhood-Onset Myasthenia Gravis Patients Treated With Tacrolimus

Zhuajin Bi[1], Yayun Cao[2], Jing Lin[1], Qing Zhang[1], Chenchen Liu[1], Mengcui Gui[1] and Bitao Bu[1]**

[1] Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2] Department of Radiology, Zhongnan Hospital of Wuhan University, Wuhan, China

OPEN ACCESS

Edited by:
Nils Erik Gilhus,
University of Bergen, Norway

Reviewed by:
Vuslat Yilmaz,
Istanbul University, Turkey
Yuwei Da,
Capital Medical University, China

***Correspondence:**
Mengcui Gui
mengcuigui@tjh.tjmu.edu.cn
Bitao Bu
bubitao@tjh.tjmu.edu.cn

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: 22 November 2021
Accepted: 10 January 2022
Published: 08 February 2022

Citation:
Bi Z, Cao Y, Lin J, Zhang Q, Liu C, Gui M and Bu B (2022) Long-Term Improvement in a Chinese Cohort of Glucocorticoid-Resistant Childhood-Onset Myasthenia Gravis Patients Treated With Tacrolimus. Front. Neurol. 13:820205. doi: 10.3389/fneur.2022.820205

Objectives: To evaluate the long-term outcome of tacrolimus for childhood-onset myasthenia gravis (CMG) with an inadequate response to glucocorticoids, and investigate factors associated with favorable outcomes following tacrolimus treatment.

Methods: A retrospective, observational cohort study was performed for CMG patients who had not improved satisfactorily after sufficient prednisone therapy for at least 8 weeks. All patients were given tacrolimus in doses of 2–3 mg for more than 6 months. The primary efficacy outcome was assessed using the prednisone dose, quantitative MG (QMG), and MG-activity of daily living (ADL) scores. The participants were divided into improved and unimproved groups based on changes in QMG scores to investigate the risk factors that affected tacrolimus efficacy.

Results: A total of 149 glucocorticoid resistant CMG patients were finally enrolled in our study, with 113 (75.8%) responding well to tacrolimus (defined as minimal manifestation status or better). One month after initiating tacrolimus, there was a noticeable improvement in prednisone dose, QMG, and ADL scores, which continued to improve throughout the study. More importantly, the prednisone was eventually stopped in 89 of the patients (78.8%). Thymus type [odds ratio (OR) = 3.156, 95% confidence interval (CI) 1.427–6.978; $P = 0.005$] and pre-intervention status (OR = 0.284, 95%CI 0.109–0.741; $P = 0.010$) were independent predictors of tacrolimus efficacy after controlling for confounding factors in multiple logistic regression.

Conclusion: The majority of glucocorticoid-resistant CMG patients have a good long-term prognosis after adding tacrolimus. Thymus type and pre-intervention status can serve as potential predictors affecting the efficacy of tacrolimus.

Keywords: myasthenia gravis, children, tacrolimus, thymus type, pre-intervention status

INTRODUCTION

Myasthenia gravis (MG) is an acquired autoimmune disorder caused by antibodies that target the neuromuscular junction, leading to extraocular and/or systemic skeletal muscle weakness and fatigability (1, 2). The age distribution in MG seems different between Caucasians and East Asian populations (3, 4). In China, there are more than half of MG patients initially developed symptoms in childhood (5). The long-term treatment methods for MG patients usually include pyridostigmine, glucocorticoids (steroids) and immunosuppressants (IS) (6, 7). However, about 20–35% of the MG patients were insensitive to steroid therapy (8, 9). Furthermore, compared with adult-onset MG (AMG), childhood-onset myasthenia gravis (CMG) is more likely to develop resistance to steroids and suffer serious adverse drug reactions (ADRs) from long-term immunotherapy (5, 8). Alternative approaches with more satisfied efficacy and less serious ADRs are urgently needed for long-term use in CMG patients. Majority of CMG patients experienced fluctuating course characterized by remitting-relapsing pattern and slowly developed unresponsiveness to pyridostigmine and corticosteroids in China (8). The long-term outcome of CMG patients remained a major concern.

Tacrolimus, a kind of immunosuppressants by inhibiting interleukin-2 production, Th1 and Th17 responses, and T lymphocyte activation (10), had been suggested to satisfactorily and safely improve the symptoms of AMG patients who were unresponsive or intolerant to steroids (11, 12). However, clinical data about the efficacy and safety of tacrolimus in CMG is very limited, due to the difficulties with study design and recruitment of patients in sufficiently large numbers (5, 11, 13–15). In this study, we evaluated the efficacy and safety of tacrolimus in a cohort of steroid-resistant CMG patients. In addition, clinical predictors associated with favorable outcomes have been analyzed.

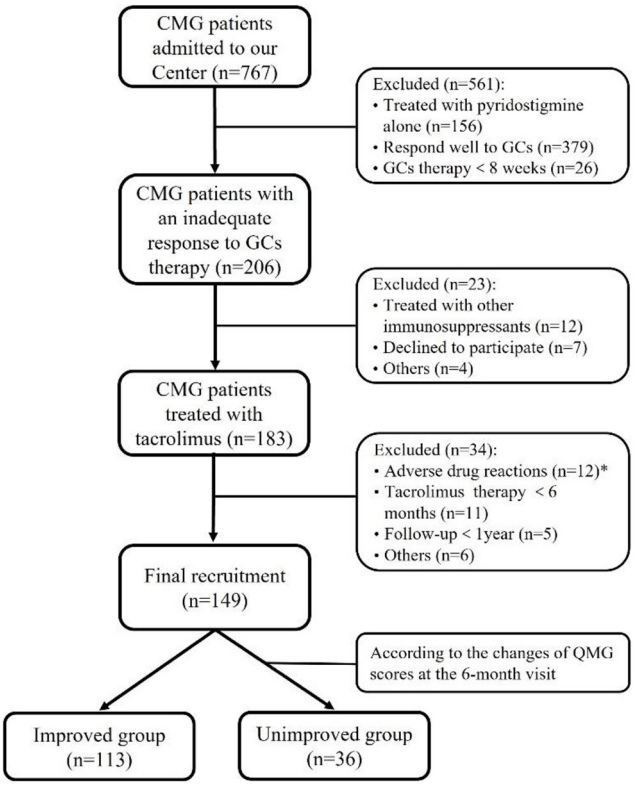


FIGURE 1 | Flowchart of participants' recruitment. *During the follow-up throughout our study, 12 out of 183 (<7.5%) patients discontinued tacrolimus due to severe adverse drug reactions after a median of 2.8 months (ranged from 0.25 to 10.00 months): renal insufficiency in three, hepatic dysfunction in two, stomachache in two, tremor in two, hyperglycemia in one, infection in one, and allergic to tacrolimus in one.

MATERIALS AND METHODS

Study Design and Patient Selection

This study is a retrospective analysis of CMG patients from a single centre. Steroids resistant CMG patients were evaluated at Tongji hospital of Tongji Medical College, Huazhong University of Science and Technology from January 2015 to May 2020. The inclusion criteria were as follows: (1) patients with a confirmed diagnosis of MG based on the fatigable weakness of the skeletal muscles and at least one of the following positive results of the neostigmine test, repetitive nerve stimulation (RNS) test, or MG-related autoantibody test; (2) patients with onset age ≤14 years; (3) patients had an inadequate response to prednisone at doses of ≥0.75 mg/kg/day for at least 8 weeks prior to enrolment. The inadequate response was defined as meeting at least one of the following criteria (11, 16): ① QMG score or MG-ADL score improved by <25%; ② the steroids dosage failed to reduce; ③ the MGFA post-intervention state (PIS) didn't improve.

Patients were excluded if they had any of the following conditions: (1) tacrolimus was not available because of complications, including diabetes, abnormal liver, and kidney function, or severe infectious diseases; (2) tacrolimus was withdrawn due to ADRs; (3) thymectomy or steroid-sparing agents were used within 3 months before the start of tacrolimus administration; (4) duration of follow-up is <1 year. In addition, to investigate factors that may potentially affect the efficacy of tacrolimus, the patients were divided into two groups based on the changes of QMG scores at the 6-month visit: the improved group with reduction of QMG score ≥25% and unimproved group with reduction of QMG score<25% (16, 17). **Figure 1** depicted the selection procedure.

Therapy, Evaluation, and Follow-Up

All participants were given 0.05 mg/kg/day of tacrolimus (Hangzhou Zhongmei Huadong Pharmaceutical Co., Ltd., H20094027), which was later adjusted to a trough level between 5 and 10 ng/dL according to the therapeutic effect (17). For patients with rapid tacrolimus metabolism, Wuzhi capsules, an ethanol herbal extract of Schisandra sphenanthera, were usually added to increased tacrolimus oral bioavailability (18). To investigate the long-term safety of tacrolimus, routine laboratory tests were performed every 4 weeks after tacrolimus administration to identify potential abnormalities in blood count,

electrolytes, serum chemistry and blood glucose. The prednisone dose was gradually reduced by 5–10 mg per month after a noticeable improvement in symptoms. After a successful steroids withdrawal, the dose of tacrolimus was reduced by 0.5 mg every 3–6 months and subsequently removed after at least 6 months of MM or better status. If the clinical symptoms recurred, the dose of prednisone or tacrolimus was increased immediately until the symptoms improved and stabilized. Because prednisone was utilized to treat the majority of subjects (83.2%) in our study, their steroid dosages were expressed as equivalents to prednisone when oral steroids other than prednisone were used.

MG is classified as ocular MG (OMG) and generalized MG (GMG) according to symptoms within the first month of onset (19). The MGFA classification was used to evaluate the maximum clinical severity before tacrolimus administration, and MGFA-PIS was used to assess the clinical status at the last visit (20). In terms of the MGFA PIS, the category of "MM or better status" included minimal manifestation (MM) status, pharmacological remission (PR), and complete stable remission (CSR). Therapeutic effects were evaluated using the dose of prednisone, MG-ADL, and QMG scores. In addition, MG-ADL and QMG scores were performed at 3–4 h after the last dose of pyridostigmine to avoid the potential influence of cholinesterase inhibitors. Follow-up was conducted to evaluate the therapeutic effect for all patients and adjust the therapeutic agents was done once a month for the first 6 months of tacrolimus treatment and at least once every 3 months after that.

Statistical Analysis

Numerical data are presented as mean ± standard deviation (SD) or median (interquartile range, IQR), and categorical data are presented as frequencies with absolute numbers and percentages. The changes in the titers of AChR-ab, prednisone dose, QMG, and ADL scores were accessed using Wilcoxon signed-rank test at each follow-up visit. Kaplan-Meier curve was used to visualize the discontinuation rate of steroids during the tacrolimus treatment. A univariate logistic regression analysis was applied to identify possible factors correlated with the efficacy of tacrolimus and entered variables with p values < 0.20 into the multivariate logistic regression analysis. Additionally, the spearman rank test was performed in all variables to reduce confounders if 2 variables have a correlation coefficient ≥ 0.5. After that, a multivariate logistic regression model was performed to determine predictors that independently affected the efficacy of tacrolimus, using a stepwise forward selection procedure with a 0.05 threshold for both inclusion and exclusion. All statistical analyses were performed with SPSS version 22.0 (SPSS Inc. Chicago, IL, USA), and two-tailed $P < 0.05$ was deemed to indicate statistical significance.

RESULTS

Demographic Characteristics

Among 767 CMG patients in our centre, 206 cases (35.2 %) were resistant to corticotherapy and 183 cases were then treated with tacrolimus. In addition, 12 out of 183 (<7.5%) patients discontinued tacrolimus due to severe ADRs after a median of 2.8 months (ranged from 0.25 to 10.00 months) (**Figure 1**). Thus, a total of 149 patients (median [IQR] age at onset: 4.4 [2.5, 7.4] years; 65.1% female) were enrolled in the study, with a follow-up for a median of 12.9 years (IQR: 6.9, 19.2) (**Table 1**). There was no significant difference in age of onset between males and females ($P = 0.866$) (**Figure 2A**). Of all patients, 140 patients (94.0%) showed only ocular symptoms at onset (MGFA class I). Ptosis was the most common initial presentation in 67.1% (100/149) of patients. 6.0% (9/149) of patients had generalized muscle weakness at onset. Besides,

TABLE 1 | Baseline characteristics of 149 study participants.

Characteristics	Patients
Gender	
Male	52 (34.9)
Female	97 (65.1)
Age at onset (years)	4.4 (2.5, 7.4)
≤ 5 years	84 (57.1)
5–10 years	42 (27.5)
> 10 years	23 (15.4)
Duration (years)	12.9 (7.4, 19.2)
Complicated with other AID	24 (16.1)
Neostigmine test (+)	144 (96.6)
RNS abnormalities	20/31
Autoantibody status[a]	
AChR-ab (+)	113/149
MuSK-ab (+)	1/84
Thymus type[b]	
Normal	82 (55.0)
Hyperplasia	59 (39.6)
Thymoma	9 (5.4)
Thymectomy	26 (17.4)
Age at thymectomy (years old)	16.0 (11.3, 20.6)
Time from onset to thymectomy (years)	12.2 (6.2, 15.7)
Ocular MG at onset	140 (94.0%)
Ptosis	100 (67.1)
Diplopia	11 (7.4)
Ptosis and diplopia	21 (14.1)
Ptosis and strabismus	8 (5.4)
Generalized MG at onset	9 (6.0%)
Limb weakness	3 (2.0)
Bulbar weakness	4 (2.7)
Limb and bullar weakness	2 (1.3)
Generalized disease development (years)[c]	12.0 (6.4, 17.2)
Within 2 years	3 (2.0)
After 2 years	30 (20.1)
Pre-intervention status	
Unchanged	31 (20.8)
Worse	24 (16.1)
Exacerbation	94 (63.1)

Data are given as n (%) or median (interquartile range).

[a] The AChR-ab titers >0.50 nmol/L and MuSK-ab titers >0.05 nmol/L were defined as positive (RIA kit, RSR Limited, Cardiff, UK).

[b] Thymus status was evaluated by chest computed tomography (CT) scan in non-thymectomized patients and thymus histology in thymectomized patients.

[c] Because only patients with ocular forms at onset can develop a generalized disease, the denominators are the number of patients with ocular forms at onset.

AChR-ab, anti-acetylcholine receptor antibodies; AID, autoimmune disease; MG, Myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; MuSK-ab, anti-muscle specific kinase autoantibody; RNS, repetitive nerve stimulation.

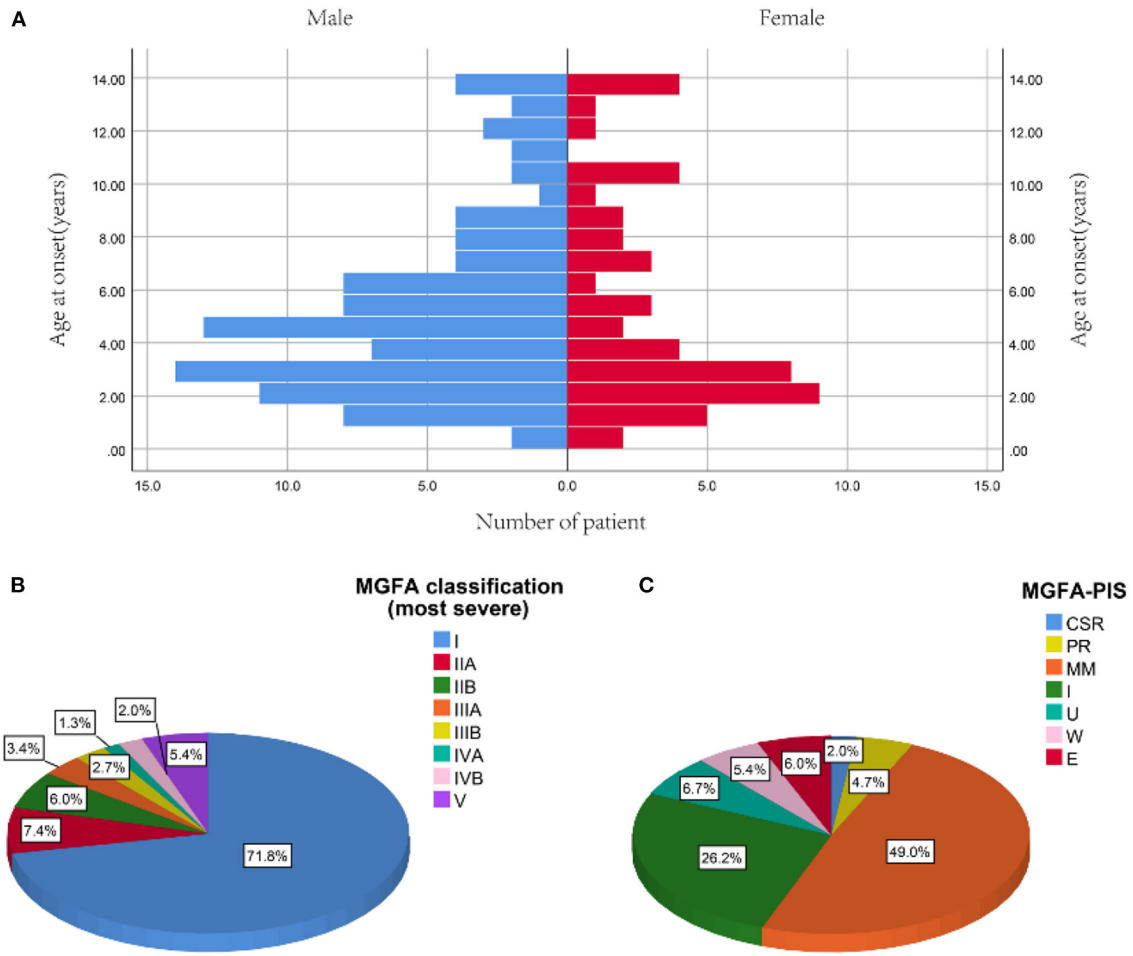


FIGURE 2 | Profiles of study participants. **(A)** Distribution of onset age between male and female. **(B)** The most severe MGFA classification before tacrolimus administration. **(C)** MGFA-PIS on the last follow-up [median 12.9 years (IQR: 6.9, 19.2) from diagnosis]. Data are presented as the number or proportion of patients in each category. CSR, complete stable remission; E, exacerbation; MGFA, Myasthenia Gravis Foundation of America; MM, minimal manifestation; PIS, post-intervention state; PR, pharmacologic remission; U, unchanged; W, worse.

33 out of the 140 OMG patients (20.4%) had transformed into GMG after a median of 12.0 years (IQR: 6.4–17.2). MG severity was classified as mild (MGFA I or II) in 127 patients (85.2%) and severe (MGFA III-V) in 22 patients (14.8%) before tacrolimus initiation (**Figure 2B**). The positive AChR-ab or MuSK-ab were detected in 113 (87.5%) patients and 1 (1.2%) patient, respectively. Thymus status was evaluated in all patients by chest computed tomography (CT) scan (2 thymomas, 39 thymus hyperplasia, 82 normal thymus) or thymus pathology (6 thymomas, 20 thymus hyperplasia). Thymectomy had been performed in 26 patients (17.4%) and the median (IQR) time from onset to thymectomy was 12.2 years (6.2, 15.7). One hundred and thirty two patients (79.0%) attained CSR, PR, MM or improvement at the last visit. However, 10 patients (6.7%) remained unchanged, 8 patients (5.4%) had clinically worsened symptoms, and 9 patients (6.0%) experienced exacerbated (**Figure 2C**).

Efficacy Evaluation of Tacrolimus

All patients received a daily dose of 2–3 mg of tacrolimus with a mean trough concentration of 5.6 ± 1.5 ng/ml. The median age at the start of the tacrolimus was 15.2 years old (IQR, 9.3–22.2), and the median disease duration before initiating tacrolimus was 9.9 years (IQR, 4.1–16.3). After a mean follow-up of 3.16 ± 1.33 years, the tacrolimus dosage had been successfully tapered from 2.53 ± 0.74 to 1.55 ± 0.66 mg/day in 16 patients and withdrawn in 8 patients without any deterioration. The remaining 125 patients needed to maintain the initial tacrolimus dose to control the symptoms.

In addition, all patients had received prednisone for a median (IQR) duration of 2.0 (0.6, 4.5) years before tacrolimus and the median (IQR) age at prednisone initiation were 8.9 (4.5, 17.3) years old. The mean prednisone dosage significantly decreased after tacrolimus was added to treatment, from 17.47 ± 9.16 mg/day at baseline to 6.42 ± 6.39 mg/day at the

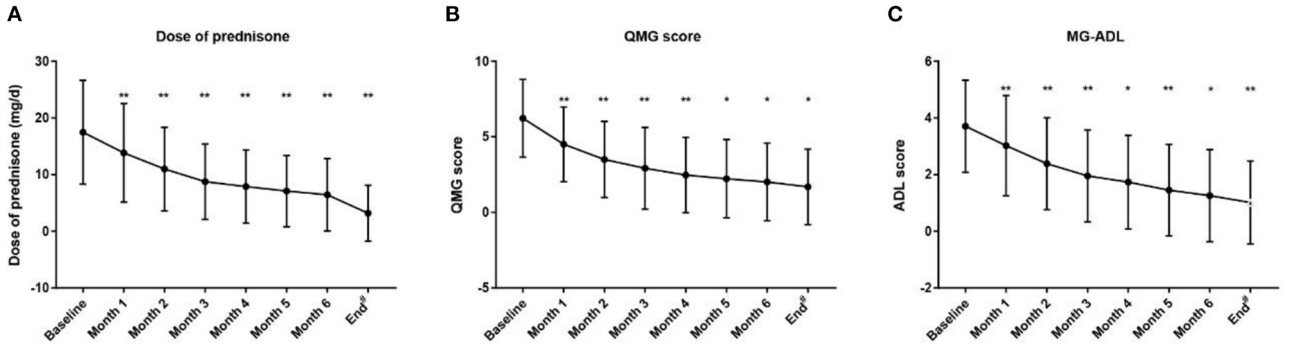


FIGURE 3 | Changes of the prednisone dose, QMG score and MG-ADL score during treatment with tacrolimus. #Mean follow-up of 3.16 ± 1.33 years (range 1.24–7.18 years). ADL, activity of daily living; QMG, quantitative MG. **(A)** Dose of prednisone, **(B)** QMG score, **(C)** MG-ADL score decreased gradually after initiation of tacrolimus treatment during the 6-month following-up (Dose of prednisone were 17.47 ± 9.16, 13.83 ± 8.69, 10.97 ± 7.38, 8.74 ± 6.65, 7.87 ± 6.46, 7.08 ± 6.28, 6.42 ± 6.39, 3.17 ± 4.93; QMG scores were 6.22 ± 2.58, 4.50 ± 2.47, 3.50 ± 2.52, 2.91 ± 2.71, 2.47 ± 2.49, 2.22 ± 2.59, 2.01 ± 2.57, 1.68 ± 2.49; and ADL scores were 3.70 ± 1.63, 3.02 ± 1.77, 2.38 ± 1.62, 1.95 ± 1.62, 1.73 ± 1.65, 1.45 ± 1.61, 1.26 ± 1.63, 1.01 ± 1.46 at the start of acrolimus treatment, 1, 2, 3, 4, 5, 6 month and the end of follow-up; respectively) (Compared with the last follow-up time point, $^{*}P < 0.05$, $^{**}p < 0.001$; 2-tailed Wilcoxon signed-rank test). $^{*}p < 0.05$. $^{**}p < 0.01$.

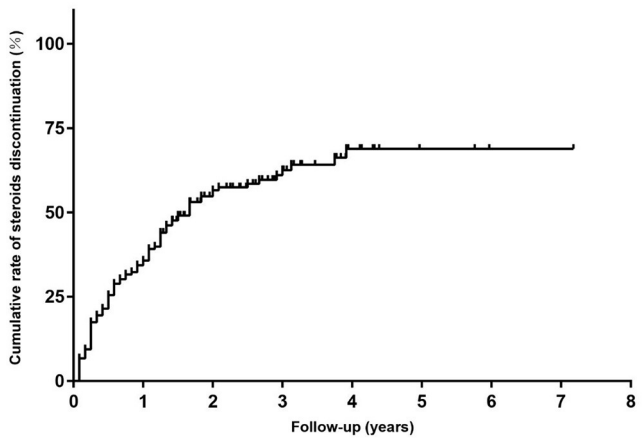


FIGURE 4 | The Kaplan-Meier curve for time to discontinued steroids in children with steroid-resistant MG.

6-month visit (**Figure 3A**, $p < 0.001$). Furthermore, at 3, 6, 12, 24, and more than 24 months of follow-up, 26, 12, 15, 28, and 8 cases were withdrawn from prednisone due to improvement following tacrolimus treatment, respectively (**Figure 4**). Compared to the baseline, there was a statistically significant improvement in QMG and ADL scores at 1, 2, 3, 4, 5, and 6 months after initiating tacrolimus (**Figures 3B,C**, $p < 0.05$).

After an average of 0.9 years (range 0.5–1.8 years) of tacrolimus treatment, 41 of 113 (36.3%) anti-AChR antibody-positive cases were retested for anti-AChR antibodies, and the mean titers of AChR-ab were significantly decreased from 4.649 ± 2.564 to 2.283 ± 1.250 nmol/L ($P < 0.001$). However, no conversion from positive to negative for AChR-ab was observed in these patients. Moreover, only 1 of the 84 (1.2%) child with MG was positive for anti-MuSK antibody. This patient was a 5 year old girl who developed ptosis and diplopia followed by progressive limbs weakness, dysphagia, and dysarthria during the pyridostigmine and prednisone treatment. Thymic hyperplasia was identified on a chest CT scan. After 3 months of tacrolimus therapy, she was improved and no longer had any symptoms. This girl gradually stopped taking prednisone, and the MuSK-ab changed from positive to negative after one year.

Factors That Might Influence the Efficacy of Tacrolimus in the Treatment of CMG

The patients were divided into two groups: improved MG group ($n = 113$) and unimproved MG group ($n = 36$). The common clinical features of the improved and unimproved patients were available in **Supplementary Table 1**. Gender, thymus type, thymectomy, the tacrolimus concentrations before tapering, and pre-intervention status were found to be associated with the clinical outcome using univariate logistic regression analysis. Although the thymectomy was found to be significant by univariate analysis, it was excluded from multivariate regression analysis due to its strong correlation with thymus type (Spearman correlation coefficient = 0.544, $P < 0.001$). Finally, multivariate logistic regression analysis indicated that thymus hyperplasia and pre-intervention status were independent predictors of tacrolimus efficacy. To be specific, thymic hyperplasia compromise therapeutic efficacy of tacrolimus compared with normal thymic (odds ratio [OR] = 3.140, 95% confidence interval [CI] =1.374–7.178; $P = 0.007$) but not thymoma (OR = 1.066, 95% CI = 0.113–10.085, $P = 0.956$). For pre-intervention status, CMG with exacerbated status had better therapeutic efficacy than those with unimproved status (OR = 0.284, 95% CI = 0.109–0.741, $P = 0.010$) (**Table 2**).

TABLE 2 | Univariate and multivariate analysis for the influencing factors of the tacrolimus efficacy.

Variable	Univariable		Multivariable	
	OR (95%CI)	*P*	OR (95%CI)	*P*
Age at onset, y	1.011 (0.912, 1.120)	0.834		
Gender (male vs. female)	0.502 (0.233, 1.079)	0.078*		
Duration, y	0.502 (0.233, 1.079)	0.533		
Complicated with other AID	0.798 (0.275, 2.316)	0.678		
Neostigmine test (+)	1.011 (0.973, 1.051)	0.576		
Symptoms at onset[a]	1.621 (0.384, 6.841)	0.511		
MGFA classification	1 [Reference]	0.470		
I	1.482 (0.514, 4.273)	0.466		
II	(0.000, 0.000)	0.999		
III	2.306 (0.364, 14.604)	0.375		
IV	3.458 (0.804, 14.868)	0.095		
V				
QMG score before tacrolimus administration	1.000 (0.864, 1.158)	0.998		
ADL score before tacrolimus administration	1.051 (0.838, 1.318)	0.668		
AChR-ab titers, nmol/L	1.430 (0.566, 3.614)	0.449		
Thymus type				
Normal	1 [Reference]	0.012**	1 [Reference]	0.022**
Hyperplasia	3.156 (1.427, 6.978)	0.005	3.140 (1.374, 7.178)	0.007
Thymoma	0.758 (0.086, 6.691)	0.803	1.066 (0.113, 10.085)	0.956
Thymectomy	2.875 (1.176, 7.024)	0.021*		
Age at Pre administration, y	1.011(0.972, 1.051)	0.587		
Interval between onset and starting Pre, y	1.012 (0.969, 1.057)	0.590		
Duration of Pre before Tac, y	1.002 (0.903, 1.110)	0.976		
Age at Tac administration, y	1.012 (0.973, 1.052)	0.566		
Interval between onset and starting Tac, y	1.015 (0.971, 1.060)	0.514		
Tac concentrations before tapering, ng/mL	1.185 (0.920, 1.526)	0.189*		
Pre-intervention status				
Unimproved	1 [Reference]	0.004**	1 [Reference]	0.007**
Worse	1.131 (0.382, 3.353)	0.824	1.130 (0.364, 3.509)	0.832
Exacerbation	0.277 (0.111, 0.695)	0.006	0.284 (0.109, 0.741)	0.010

[a]*Patients were categorized as ocular MG (MGFA class I) and generalized MG (MGFA class II-V) according to the initial symptoms.*

AChR-ab, acetylcholine receptor antibody; AID, autoimmune disease; MG, Myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; OR, odds ratio; Pre, prednisone; SD, standard deviation; Tac, tacrolimus.

*$p < 0.20$; **$p < 0.05$.*

DISCUSSION

In this study, we reported the clinical data from CMG patients who did not achieve satisfactory therapeutic effects and then were treated with tacrolimus. The majority of subjects can respond well to tacrolimus, and nearly half attained MMS or better status at the end of follow-up (21). In contrast to previous studies, which found no gender bias in CMG patients in Asian populations (8, 22), our data showed a female preponderance, which was significantly lower than that the entire population of CMG patients treated at our center (seen in the **Supplementary Table 2**). This gender bias may be the result of a combination of sex hormones and genetic predisposition on the immunological function, and it represents disparities in therapeutic response to steroids between males and females (23). Similar to earlier studies that the majority of CMG patients only had ocular symptoms (8, 22, 24), the current study showed that 94.0% of recruited subjects had OMG. However, our cohorts had a higher rate of generalized conversion or severe MG (MGFA III-V) during pyridostigmine and steroids therapy. Therefore, alternative non-steroidal immune-suppressants with better efficacy is urgently needed to treat CMG patients with an inadequate response to steroids.

The QMG and MG-ADL scores were primarily used to assess the therapeutic efficacy of MG (20). Our findings revealed that these two markers began to improve 1 month after initialing tacrolimus and steadily improved throughout the study. This is in line with recent studies reporting that the therapeutic effect of tacrolimus can be shown within 4 weeks of commencing therapy, which is faster than other traditional IS, such as azathioprine, cyclophosphamide, and methotrexate (25–27). Another benefit of tacrolimus for patients with MG was that it allowed them to reduce their steroid dosages, however, there is a lot of individual variance (1, 28–30). Previous studies have demonstrated that the early favorable outcomes of tacrolimus may be caused by improving both the transport of steroids into the nucleus and the ability of steroid receptor to bind hormone (12, 29, 31). In this study, more than half patients were able to stop taking steroids after responding well to tacrolimus, while 21.2% patients needed a combination of low-doses steroids to keep their symptoms under control at the end of follow-up. Furthermore, previous studies have reported positive results with tacrolimus

monotherapy, suggesting that tacrolimus can be used alone or in combination with steroid (21, 32).

AChR antibodies were found in the majority of the participants in our study, and the reduction of AChR-ab titers was accompanied by the clinical improvement after tacrolimus treatment, which was consistent with prior researches (12, 24, 29). By comparing the AChR-ab titers before enrollment between the improved and unimproved group, we were able to show that it was not an independent risk factor for tacrolimus efficacy. These findings suggest that, while AChR-ab levels do not correlate with tacrolimus efficacy, dynamic changes in AChR-ab titers are helpful to assess the symptom improvement and guide further treatment. In addition, MG patients with MuSK-ab (MuSK-MG) had a substantially greater probability of failure with traditional IS agents compared to patients with AChR-MG (10, 33). We effectively treated a severe generalized MuSK-ab-positive CMG patient with tacrolimus in our study, suggesting that tacrolimus might be a viable treatment for children with MuSK-MG (10).

Two clinical predictors of tacrolimus efficacy were identified statistically in our study: thymus type and pre-intervention status. Although the relationship between the thymus gland and MG is not yet fully understood (34). The thymus is thought to play an important role in the pathogenesis of MG. Our data showed that concomitant thymus hyperplasia was an independent risk factor for poor efficacy of tacrolimus in children with steroid-resistant MG, even in situation when thymectomy therapy had been used. One theory is that autoreactive T-lymphocytes exported from the aberrant germinal center in thymus hyperplasia might have remained in the periphery for a long period and then been activated to disrupt immunological homeostasis (35). However, it should be noted that thymus status was mostly assessed by CT or MRI scans in our study, which may have limited sensitivity for thymus hyperplasia and hence bias of the assessment of tacrolimus effectiveness (36). Furthermore, the pre-intervention state of the improved and unimproved group differed, indicating that patients with exacerbated status before enrollment were more likely to respond effectively to tacrolimus than those with unimproved status. In terms of MGFA pre-intervention state, the majority of exacerbated patients who had previously achieved MM or better status with steroids therapy, tended to develop acquired resistance to steroids; whereas the unimproved cases did not respond to steroids once steroid therapy was initiated. This might mean that patients with MG who had developed resistance to steroids had a greater response to tacrolimus than those who had an initially poor response to steroids. Additionally, a recent cohort study reported tacrolimus combined with steroids can improve clinical effectiveness and serve as medication maintenance to prevent disease relapses in MG patients (36). Finally, because many clinical factors did not correlate with tacrolimus efficacy in steroid-resistant CMG patients, future researches should focus on biochemical and immunological indicators like NF-κB transcriptional activity, FK506-binding proteins (FKBPs), and abnormal T cell selection and activation (30, 33).

Even when the symptoms had been adequately controlled, most patients in our study were hesitant to stop taking tacrolimus because of the protracted illness course and significant chance of relapse (37). Tacrolimus dosage was successfully tapered in 24 (21.2%) of the 113 well-controlled patients in our cohorts without exacerbating their condition. In contrast to other studies which reported a higher incidence of tacrolimus-related ADRs, ranging from 42.5–87.5% (1, 11, 28, 38). Our data showed that <7.5% patients had ADRs after an average follow-up of 3 years, which may be related to the use of lower doses of tacrolimus. All the ADRs occurred within 10 months of tacrolimus treatment and were resolved when tacrolimus was discontinued. Therefore, we conclude that long-term tacrolimus usage in children with steroid-resistant MG is relatively safe.

In conclusion, children with steroid-resistant MG displayed distinct clinical characteristics. Although tacrolimus improved symptoms in the majority of steroid-resistant CMG patients with few adverse effects, some patients still did not react well to tacrolimus. Clinically independent factors affecting tacrolimus efficacy include thymus hyperplasia and pre-intervention status. And we are currently working on a follow-up study to explore the underlying immunological mechanism of therapeutic failure in patients who haven't responded to steroids or tacrolimus.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Committee of Clinical Investigation at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan China (NO. TJ-IRB20190414). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

ZB: acquired the data and drafted the manuscript. YC, JL, and QZ: interpreted the data and made suggestions for improvement. MG and BB: designed the study and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the National Natural Science Foundation of China (Grant No: 8187051428).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.820205/full#supplementary-material

REFERENCES

1. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. *Neurology*. (2016) 87:419–25. doi: 10.1212/WNL.0000000000002790
2. Dalakas MC. Immunotherapy in myasthenia gravis in the era of biologics. *Nat Rev Neurol*. (2019) 15:113–24. doi: 10.1038/s41582-018-0110-z
3. He D, Zhang H, Xiao J, Zhang X, Xie M, Pan D, et al. Molecular and clinical relationship between live-attenuated Japanese encephalitis vaccination and childhood onset myasthenia gravis. *Ann Neurol*. (2018) 84:386–400. doi: 10.1002/ana.25267
4. Zhang X, Yang M, Xu J, Zhang M, Lang B, Wang W, et al. Clinical and serological study of myasthenia gravis in HuBei Province, China. *J Neurol Neurosurg Psychiatry*. (2007) 78:386–90. doi: 10.1136/jnnp.2006.100545
5. Liu C, Gui M, Cao Y, Lin J, Li Y, Ji S, et al. Tacrolimus improves symptoms of children with myasthenia gravis refractory to prednisone. *Pediatr Neurol*. (2017) 77:42–47. doi: 10.1016/j.pediatrneurol.2017.08.014
6. Yoshikawa H, Kiuchi T, Saida T, Takamori M. Randomised, double-blind, placebo-controlled study of tacrolimus in myasthenia gravis. *J Neurol Neurosurg Psychiatry*. (2011) 82:970–7. doi: 10.1136/jnnp-2011-300148
7. Morren J, Li Y. Maintenance immunosuppression in myasthenia gravis, an update. *J Neurol Sci*. (2020) 410:116648. doi: 10.1016/j.jns.2019.116648
8. Gui M, Luo X, Lin J, Li Y, Zhang M, Zhang X, et al. Long-term outcome of 424 childhood-onset myasthenia gravis patients. *J Neurol*. (2015) 262:823–30. doi: 10.1007/s00415-015-7638-2
9. Imai T, Suzuki S, Nagane Y, Uzawa A, Murai H, Utsugisawa K. Reappraisal of oral steroid therapy for Myasthenia Gravis. *Front Neurol*. (2020) 11:868. doi: 10.3389/fneur.2020.00868
10. Li Y, Guptill JT, Russo MA, Massey JM, Juel VC, Hobson-Webb LD, et al. Tacrolimus inhibits Th1 and Th17 responses in MuSK-antibody positive myasthenia gravis patients. *Exp Neurol*. (2019) 312:43–50. doi: 10.1016/j.expneurol.2018.11.006
11. Zhou L, Liu W, Li W, Li H, Zhang X, Shang H, et al. Tacrolimus in the treatment of myasthenia gravis in patients with an inadequate response to glucocorticoid therapy: randomized, double-blind, placebo-controlled study conducted in China. *Ther Adv Neurol Disord*. (2017) 10:315–25. doi: 10.1177/1756285617721092
12. Shimojima Y, Matsuda M, Gono T, Ishii W, Tokuda T, Ikeda S. Tacrolimus in refractory patients with myasthenia gravis: coadministration and tapering of oral prednisolone. *J Clin Neurosci*. (2006) 13:39–44. doi: 10.1016/j.jocn.2004.12.008
13. Ning YM, Sánchez ER. Potentiation of glucocorticoid receptor-mediated gene expression by the immunophilin ligands FK506 and rapamycin. *J Biol Chem*. (1993) 268:6073–6. doi: 10.1016/S0021-9258(18)53220-8
14. Mori T, Mori K, Suzue M, Ito H, Kagami S. Effective treatment of a 13-year-old boy with steroid-dependent ocular myasthenia gravis using tacrolimus. *Brain Dev*. (2013) 35:445–8. doi: 10.1016/j.braindev.2012.06.012
15. Kakisaka Y, Haginoya K, Yokoyama H, Ishitobi M, Wakusawa K, Sato I, et al. Successful treatment of a 2-year-old girl with intractable myasthenia gravis using tacrolimus. *Brain Dev*. (2006) 28:534–6. doi: 10.1016/j.braindev.2006.02.001
16. Liu GC, Gao BL, Yang HQ, Qi GY, Liu P. The clinical absolute and relative scoring system-a quantitative scale measuring myasthenia gravis severity and outcome used in the traditional Chinese medicine. *Complement Ther Med*. (2014) 22:877–86. doi: 10.1016/j.ctim.2014.08.003
17. Cruz JL, Wolff ML, Vanderman AJ, Brown JN. The emerging role of tacrolimus in myasthenia gravis. *Ther Adv Neurol Disord*. (2015) 8:92–103. doi: 10.1177/1756285615571873
18. Zhang H, Bu F, Li L, Jiao Z, Ma G, Cai W, et al. Prediction of drug-drug interaction between tacrolimus and principal ingredients of wuzhi capsule in chinese healthy volunteers using physiologically-based pharmacokinetic modelling. *Basic Clin Pharmacol Toxicol*. (2018) 122:331–40. doi: 10.1111/bcpt.12914
19. Ding J, Zhao S, Ren K, Dang D, Li H, Wu F, et al. Prediction of generalization of ocular myasthenia gravis under immunosuppressive therapy in Northwest China. *BMC Neurol*. (2020) 20:238. doi: 10.1186/s12883-020-01805-1
20. Jaretzki A III, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. *Neurology*. (2000) 55:16–23. doi: 10.1212/WNL.55.1.16
21. Yagi Y, Sanjo N, Yokota T, Mizusawa H. Tacrolimus monotherapy: a promising option for ocular myasthenia gravis. *Eur Neurol*. (2013) 69:344–5. doi: 10.1159/000347068
22. Huang X, Li Y, Feng H, Chen P, Liu W. Clinical characteristics of juvenile myasthenia gravis in Southern China. *Front Neurol*. (2018) 9:77. doi: 10.3389/fneur.2018.00077
23. Evoli A. Acquired myasthenia gravis in childhood. *Curr Opin Neurol*. (2010) 23:536–40. doi: 10.1097/WCO.0b013e32833c32af
24. Hong Y, Skeie GO, Zisimopoulou P, Karagiorgou K, Tzartos SJ, Gao X, et al. Juvenile-onset myasthenia gravis: autoantibody status, clinical characteristics and genetic polymorphisms. *J Neurol*. (2017) 264:955–62. doi: 10.1007/s00415-017-8478-z
25. Tindall RS, Phillips JT, Rollins JA, Wells L, Hall K. A clinical therapeutic trial of cyclosporine in myasthenia gravis. *Ann N Y Acad Sci*. (1993) 681:539–51. doi: 10.1111/j.1749-6632.1993.tb22937.x
26. Pasnoor M, He J, Herbelin L, Burns TM, Nations S, Bril V, et al. A randomized controlled trial of methotrexate for patients with generalized myasthenia gravis. *Neurology*. (2016) 87:57–64. doi: 10.1212/WNL.0000000000002795
27. Palace J, Newsom-Davis J, Lecky B. A randomized double-blind trial of prednisolone alone or with azathioprine in myasthenia gravis. Myasthenia Gravis Study Group. *Neurology*. (1998) 50:1778–83. doi: 10.1212/WNL.50.6.1778
28. Zhao CB, Zhang X, Zhang H, Hu XQ, Lu JH, Lu CZ, et al. Clinical efficacy and immunological impact of tacrolimus in Chinese patients with generalized myasthenia gravis. *Int Immunopharmacol*. (2011) 11:519–24. doi: 10.1016/j.intimp.2010.12.012
29. Ponseti JM, Gamez J, Azem J, López-Cano M, Vilallonga R, Armengol M. Tacrolimus for myasthenia gravis: a clinical study of 212 patients. *Ann N Y Acad Sci*. (2008) 1132:254–63. doi: 10.1196/annals.1405.000
30. Erlejman AG, De Leo SA, Mazaira GI, Molinari AM, Camisay MF, Fontana V, et al. NF-κB transcriptional activity is modulated by FK506-binding proteins FKBP51 and FKBP52: a role for peptidyl-prolyl isomerase activity. *J Biol Chem*. (2014) 289:26263–76. doi: 10.1074/jbc.M114.582882
31. Li JW, Fang F, Ren XT, Zhang WH, Yang XY, Ren CH, et al. Clinical effect of tacrolimus in the treatment of myasthenia gravis in children. *Zhongguo Dang Dai Er Ke Za Zhi*. (2020) 22:964–9. doi: 10.7499/j.issn.1008-8830.2004215
32. Fan Z, Li Z, Shen F, Zhang X, Lei L, Su S, et al. Favorable effects of tacrolimus monotherapy on myasthenia gravis patients. *Front Neurol*. (2020) 11:594152. doi: 10.3389/fneur.2020.594152
33. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis - autoantibody characteristics and their implications for therapy. *Nat Rev Neurol*. (2016) 12:259–68. doi: 10.1038/nrneurol.2016.44
34. Marx A, Pfister F, Schalke B, Saruhan-Direskeneli G, Melms A, Ströbel P. The different roles of the thymus in the pathogenesis of the various myasthenia gravis subtypes. *Autoimmun Rev*. (2013) 12:875–84. doi: 10.1016/j.autrev.2013.03.007
35. Fujii Y. Thymus, thymoma and myasthenia gravis. *Surg Today*. (2013) 43:461–6. doi: 10.1007/s00595-012-0318-2
36. Zhang C, Bu B, Yang H, Wang L, Liu W, Duan RS, et al. Immunotherapy choice and maintenance for generalized myasthenia gravis in China. *CNS Neurosci Ther*. (2020) 26:1241–54. doi: 10.1111/cns.13468
37. Nishida Y, Takahashi YK, Kanai T, Nose Y, Ishibashi S, Sanjo N, et al. Safety of tapering tacrolimus dose in patients with well-controlled anti-acetylcholine receptor antibody-positive myasthenia gravis. *Eur J Neurol*. (2020) 27:100–4. doi: 10.1111/ene.14039

38. Kim YH, Shin HY, Kim SM. Long-term safety and efficacy of tacrolimus in myasthenia gravis. *Yonsei Med J.* (2019) 60:633–9. doi: 10.3349/ymj.2019.60.7.633

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Neurology

REVIEW
published: 23 February 2022
doi: 10.3389/fneur.2022.834212

The Epidemiology and Phenotypes of Ocular Manifestations in Childhood and Juvenile Myasthenia Gravis: A Review

Jeannine M. Heckmann[1,2]*, *Tarin A. Europa*[1,2], *Aayesha J. Soni*[1] and *Melissa Nel*[2]

[1] Division of Neurology, Department of Medicine, University of Cape Town, Cape Town, South Africa, [2] Neurology Research Group, University of Cape Town (UCT) Neuroscience Institute, University of Cape Town, Cape Town, South Africa

Myasthenia gravis (MG) appears to have a similar incidence among adult populations worldwide. However, epidemiological and phenotypic differences have been noted among children and juveniles with MG. We reviewed the literature on childhood- and juvenile-onset MG among different populations, with the focus on ocular involvement, antibody profiles, the genetic susceptibility to juvenile MG phenotypes, the use of immune treatments, and the reported responses of extraocular muscles to therapies. Although epidemiological studies used different methodologies, reports from Asia, compared to Europe, showed more than two-fold higher proportions of prepubertal onset (before 12 years) vs. postpubertal-onset juveniles with MG. Compared to European children, ocular MG was 4-fold more frequent among Asian children, and 2–3-fold more frequent among children with African ancestry both in prepubertal and postpubertal ages at onset. These results suggest genetic influences. In Asia, *HLA-B*46* and *DRB1*09* appeared overrepresented in children with ocular MG. In Europe, children with MG had a significantly higher rate of transforming from ocular to generalized disease and with an overrepresentation of *HLADRB1*04*. Although treatment regimens vary widely and the responses to immune therapies of the ocular muscles involved in MG were generally poorly described, there were indications that earlier use of steroid therapy may have better outcomes. Reports of treatment-resistant ophthalmoplegia may be more frequent in African and Asian juvenile MG cohorts compared to Europeans. Genetic and muscle gene expression studies point to dysregulated muscle atrophy signaling and mitochondrial metabolism pathways as pathogenetic mechanisms underpinning treatment-resistant ophthalmoplegia in susceptible individuals. In conclusion, phenotypic differences in juveniles with ocular manifestations of MG were evident in different populations suggesting pathogenetic influences. Treatment responses in MG-associated ocular disease should attract more careful descriptive reports. In MG, extraocular muscles may be vulnerable to critical periods of poor force generation and certain individuals may be particularly susceptible to developing treatment-resistant ophthalmoplegia. The development of prognostic biomarkers to identify these susceptible individuals is an unmet need.

Keywords: treatment refractory ophthalmoplegia, ocular myasthenia gravis (OMG), childhood myasthenia gravis, juvenile myasthenia gravis, genetic susceptibility, Asian ancestry, myasthenia (myasthenia gravis—MG), African ancestry

OPEN ACCESS

Edited by:
Hai-Feng Li,
Capital Medical University, China

Reviewed by:
Chongbo Zhao,
Fudan University, China
Lorenzo Maggi,
IRCCS Carlo Besta Neurological Institute Foundation, Italy

***Correspondence:**
Jeannine M. Heckmann
jeanine.heckmann@uct.ac.za

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *13 December 2021*
Accepted: *24 January 2022*
Published: *23 February 2022*

Citation:
Heckmann JM, Europa TA, Soni AJ and Nel M (2022) The Epidemiology and Phenotypes of Ocular Manifestations in Childhood and Juvenile Myasthenia Gravis: A Review. Front. Neurol. 13:834212. doi: 10.3389/fneur.2022.834212

BACKGROUND

Myasthenia gravis (MG) represents a heterogeneous group of autoantibody-mediated diseases targeting the neuromuscular junction. Extraocular muscles (EOMs) are highly susceptible to manifesting myasthenic weakness and are frequently involved early in the MG disease course prior to developing generalized myasthenia (1, 2). Younger children appear to have a higher prevalence of developing ocular MG (myasthenia remains confined to the EOMs for an extended period) among Asian cohorts, but the outcomes of EOMs to MG therapies are generally not adequately described. This study aimed to review the epidemiological literature of childhood and juvenile MG and determine the severity of ocular phenotypes and treatment responses, as well as current postulates related to the pathogenetic mechanisms underlying the ocular phenotypes with the focus on, but not confined to, the past decade.

METHODS

Search Strategy and Selection Criteria

Epidemiological Data

We searched the PubMed database for reports published in English between January 1, 2010 and October 31, 2021 with the MeSH terms "juvenile" or "childhood" in combination with "myasthenia gravis," "ocular myasthenia gravis," and "antibody." We also selected references from manual searches of reference lists of articles and reviews. Some of these references were published before 2010, but after 1991. We included publications that had clearly stated diagnostic criteria and in which epidemiological data could be extracted such as age at onset and phenotypic characteristics such as acetylcholine receptor (AChR)-antibody status, ocular involvement, secondary generalization, frequency of autoimmune diseases and thymoma, sex differences, and outcomes of ocular myasthenia. If there were 2 publications from the same group, then we included only the most recent article unless unique data was mentioned in the first report. For juvenile MG, we included reports specifying age at onset of MG symptoms between 1 and 20 years, despite the most frequent age cutoff for juvenile onset MG being < 18 years (3).

Genetic Data

Search terms included; "gene" or "HLA" and "ocular myasthenia," "juvenile myasthenia gravis," "childhood myasthenia gravis," and "extraocular muscles." We also searched using geographical terms "Asia," "China," "Africa," and "myasthenia gravis." Original research articles written in English and published between 1996 and October 2021, which compared MG/MG subgroup vs. age and race matched healthy controls, were selected for review particularly if there was a special reference to EOM involvement at presentation, treatment approaches, and descriptive outcomes to treatment. When appropriate data were extracted for positive individual human leukocyte antigen (HLA) associations (excluding haplotypes) with MG (by subgroup if specified).

Data Extraction and Organization

Although the use of critical appraisal tools to judge the scientific merit of studies for inclusion in a review is encouraged, the scarcity of studies including adequate descriptions of ocular manifestations made the use of such tools difficult to implement. A further limitation was the heterogeneity of age cutoffs for juveniles, childhood, prepubertal and postpubertal cases with MG; while most reports define the age of 12 years as the threshold of puberty and < 18 years as juvenile onset, there were different cutoffs to differentiate postpubertal MG from early-onset adult MG, and childhood MG from prepubertal MG. These were indicated as per author(s) and grouped together for comparative purposes (**Table 1**).

RESULTS

Population Differences in MG by age at Symptom Onset

Although there is recognition worldwide of an increasing predominance of MG among the elderly, including in Asia and Africa (4, 21–25), incidence rates among younger people manifesting with MG appear to differ between Asia and Europe. Population data including children are sparse and methodologies vary widely, but there appear to be four-fold higher incidence rates of MG among younger children from Asia compared to Europe and North America (12, 19, 26). A multiracial pediatric cohort from the United Kingdom (UK) in which data were accrued over 10 years showed similar findings with higher proportions of Afro-Caribbean, Asian, and Arabic children with MG compared to Caucasian children living in the UK (20).

Reports from China regarding the proportions of juveniles with MG, vary substantially and ranged between 27% (302/1,108) in northern China, and 45% (964/2,154) in southern China (27) (**Table 1**). Nevertheless, at least half of the children manifested with MG before the age of 10, and the incidence peaked in those presenting with symptoms before the age of 5 years (6, 8) (**Table 1**). A nationwide MG prevalence questionnaire from Japan showed that children developing MG before the age of 10 years accounted for 9% of the overall proportion of MG cases ($n = 3{,}061$) (4), which is much lower compared to China, but remains substantially higher than the 2% prevalence in Italy (13). Therefore, despite the possible impact of differences in study methodology on the epidemiological results, the incidence of MG in both the prepubertal and postpubertal juveniles, compared to adult-onset disease, was lower in juveniles with European genetic ancestry compared to those with Asian and African genetic ancestry.

Population and Phenotype Differences Among Categories of Juveniles With MG

Prepubertal vs. Postpubertal Onset

There is accumulating evidence that MG presenting in the prepubertal phase in contrast to postpubertal onset differs by genetic ancestry. Studies from Asia showed the proportions of children developing myasthenia before puberty ($\geq$74%) were more than twice as high compared to postpubertal children, and

TABLE 1 | Characteristics of juvenile myasthenia gravis (MG) and subgroups (pre-pubertal vs. post-pubertal) by race and/or geographical area.

References	Region		Pre-pubertal MG			Post-pubertal MG			JMG
		N	AAO (%)	AChR+	OMG	AAO (%)	AChR+	OMG	Thymoma
Asian and Indian ancestry juveniles									
Murai et al. (4)	Japan	268	<10	≈50%	62–81%	NR	NR	NR	4–10%
Gui et al. (5)	China	424*	≤10 (86%)	≈70%	≈95%	10–14 (14%)	≈70%	≈95%	17%
Feng et al. (6)	China South	130	<10	58%	NR	10–19	42%	NR	NR
Lee et al. (7)	South Korea	88	<12 (74%)	90%	97%	12–18 (26%)	87%	70%	NR
Wang et al. (8)	China North	302	<5 (50%)	NR	73%	5–15 (≈50%)	NR	66%	NR
Cohorts with >40% African ancestry juveniles									
Xu et al. (9)	USA (Texas)	60	<10 (40%)	NR	58%	10–17 (60%)	NR	14%	NR
Barraud et al. (10)	France	40	<12 (48%)	58%	37%	12–18 (52%)	NR	24%	2%
Heckmann et al. (11)	South Africa	190	<12 (41%)	56%	43%	12–20 (69%)	NR	NR	1–3%
Cohorts with >45% European ancestry juveniles									
VanderPluym et al. (12)	Canada**	49	≤12 (80%)	52%	46%	13–17 (20%)	≈90%	0	NR
Evoli et al. (13)	Italy	19	<10	74%	26%	NR	NR	NR	0%
Popperud et al. (14)	Norway	63	<12 (33%)	57%	14%[#]	12–18 (67%)	83%	12%	0%
Jastrzebska et al. (15)	Poland	101	<12 (15%)	71%	NR	12–18 (85%)	94%	NR	1%
Juvenile MG									
Wong et al. (16)	Hong Kong	101	–	–	–	<16	ND	71%	8%
Chou et al. (17)	Taiwan	54	–	–	–	<20	57%	78%	2%
Ashraf et al. (18)	India	77	–	–	–	<15	[##]	27%	1%
Mansukhani et al. (19)	USA	217	–	–	–	<19	83%	23%	0%
Vecchio et al. (20)	UK	74	–	–	–	<16	84%	51%	NR

Inclusion into this table required some demographic details according to the columns. AAO refers to the age at symptoms onset (in years as indicated by the respective authors) and % refers to the proportion of the juvenile sample satisfying the prepubertal or postpubertal definition (as indicated for each study) if available; N, refers to sample size; NR, nor reported; ND, not done; OMG refers to ocular MG (for this review, persistence of ocular only symptoms >1 year); JMG, juvenile MG; AChR+ refers to those with detectable antibodies to the acetylcholine receptor.

**5 years of follow-up required for inclusion; **48% of cohort European and 28% Asian ancestries; – indicates incomplete data for prepubertal vs. postpubertal, therefore presented data as juvenile MG. [#]used the follow-up data. [##]AChR+ data only available for 18% (11/14 AChR+).*

contrasts with a more even distribution (∼40 to 48%) amongst cohorts with African children, and <33% in cohorts comprising European children (**Table 1**). A large cohort from China showed that half of the juveniles developing MG before age 15 were younger than 5 years (8).

In Asia, there was a definite tendency toward more ocular MG amongst the very young, prepubertal children compared to older aged children with MG, but this was not evident in the Norwegian children (**Table 1**). A multiracial juvenile MG cohort from Canada, in which only 48% had European ancestry, also showed a much higher proportion of prepubertal onset MG, and most of the very young onset ocular MG cases (aged ≤ 6 years) had Asian ancestry (12).

Interestingly, two multiracial cohorts from France (48% of 40 had African ancestry) (10) and the UK (54% of 74 did not have European ancestry) (20) showed similar results in which prepubertal ocular MG were more likely in the African children despite equal proportions of children with pre- and postpubertal MG. A feature of MG among north European children (Norway and Italy) was that ocular only presentations of MG occurred in less than a third, with most children (>75%) developing generalized disease (with/or without respiratory involvement) within 2 years, and between 15 and 26% remained with ocular MG (13, 14). Similar observations were noted in Canada where white children were more likely to develop generalized MG, and Asian children remained with ocular disease (12). Furthermore, the conversion of ocular MG cases to generalized disease was reported in only 5 to 20% of Chinese and Thai children (5, 16, 28, 29) and among 25% of the French cohort in which almost half the children had African genetic ancestry (10).

Sex differences and severity of MG were not consistently different in postpubertal cohorts from different populations; a European cohort showed more girls in the postpubertal group with less severe MG disease (14); two Asian cohorts showed similar proportions of girls and boys, but inconsistent severity of MG grades by sex were reported (7, 28). An older study from the USA, which specifically assessed MG outcomes by race in a clinical setting where the same treatment approaches were used for all children, reported infrequent clinical remissions in prepubertal black patients compared to white patients, although overall disease severity was similar irrespective of race (30). It is important to highlight that MG crises can occur in children and require appropriate immune therapies (3, 12, 26).

In summary, pre- and postpubertal MG cases were more likely to remain confined to the ocular muscles in Asian children compared to those in Europe.

Antibody Profile

The AChR-Ab positive MG frequencies by RIA appeared to be similar in all children and in almost all studies ranged between 50 and 95%, irrespective of whether the MG onset was prepubertal or postpubertal (**Table 1**). A study from China found similar proportions of AChR-Abs by RIA and cell-based assay (CBA) in juveniles (<19 years of age) compared to adult-onset MG cases, although 18% of the juveniles (compared to 10% of adults) were only positive by CBA (31). The age-adjusted incidence rates of AChR-Ab positive MG among juveniles from South Africa (24) appeared to be higher than in Caucasian cohorts from the UK, USA, Norway, and Canada (≈3 per million vs. < 1.5 per million, respectively) (12, 19, 32, 33).

Data on the prevalence of muscle-specific kinase (MuSK)-Abs are sparse. Only rare cases of MuSK-Ab positive MG have been reported in juveniles from China [0/118 (31) or < 3% (6)], Japan (1.4%) (4), North America (34), and northern Europe (35) and possibly more than expected in the two cohorts with African ancestry children (10, 14, 15, 17, 19, 20).

Overall, most populations reported that younger children were more likely to have AChR-Ab negative MG and ocular disease, both of which conferred a higher likelihood of obtaining remission status (17, 20, 28). However, in the situation where the child does not respond to treatment, despite symptom onset after infancy, the question of possible congenital myasthenia may arise. Clinical features supportive of autoimmune MG include: subacute progressive onset; marked asymmetry of ptosis; substantial fluctuations of ophthalmoplegia (36).

Thymoma Incidence

Thymoma occurs rarely in juveniles with MG (34). Data from Asia varied between 0 (0/118) (31) and 17% (6/34) (**Table 1**).

Autoimmune Disease

Concomitant autoimmune disease, mainly thyroid disease, was reported in 4–19% of children with MG from China, Thailand, Hong Kong, and racially diverse cohorts from Canada and the UK (4, 5, 12, 16, 20, 27–29), 27% from Taiwan (included MG onset before age 20) (17) and ≈7% in juvenile cohorts with substantial African ancestry MG cases (10, 11). In contrast, ≈30% of pre- and postpubertal Norwegian children had other autoimmune diseases in addition to MG (14).

Epidemiology of Ocular MG Among Juveniles

The higher frequencies of ocular MG among younger children from Asia differed substantially from Europe (4, 22, 28) (**Table 1**). Within the prepubertal onset range, the very young children presenting with symptoms before the age of 4, showed the highest proportions of ocular MG compared to older children from China and Japan (4, 23, 28). African, Afro-Caribbean, and African-American prepubertal onset children also showed higher proportions of ocular MG compared to postpubertal juveniles (9, 11, 12, 20).

Severity of Extraocular Muscle Involvement at the Presentation of MG in Juveniles vs. Adults

There was a paucity of descriptive data of EOM involvement in MG. An audit of the examination findings in adults presenting with MG to a Scottish ophthalmological service, reported bilateral weakness of multiple EOMs in more than half the patients, irrespective of age, with 6% having bilateral ophthalmopareses (or duction failure) (37). A review from Thailand, but in juveniles (<15 years) presenting with ocular MG, also found limitations of EOM movement in more than 50% (of 62), and most had complete duction failure (29). Juvenile MG cases seen at the Mayo clinic (most were Caucasian children) found limitations of EOM movement in 30%, although there may be a bias to more severe cases in this cohort as most patients were not residents of the county (19).

Observational descriptive EOM data from a largely adult MG clinic, prior to any therapy and in which ≈15% had only ocular manifestations of MG, showed that ≈12% of MG cases had fatigable ptosis/diplopia compared to ≈87% with persistent ophthalmoparesis (or weakness) with or without ptosis in at least one EOM (38). Of those with ophthalmoparesis, > 60% had weakness of ≥ 6 EOMs. There was a trend toward more severe weakness in those with generalized MG compared to ocular only MG (severity is defined by the number of EOMs with ≥ 50% weakness (i.e., can only move half of the EOM's full trajectory) (38). It is worth mentioning that even mild weakness of one EOM may cause diplopia, and those patients with complete ophthalmoplegia may not experience diplopia, although minor malignment of the visual axes may result in diplopia (39).

Taken together, a substantial proportion of patients with MG may develop persistent weakness of their EOMs (ophthalmoparesis or ophthalmoplegia), and this may occur more frequently in juveniles. However, the absence of a standardized approach to reporting does not allow for firm conclusions (see below).

Treatment Outcomes of Extraocular Muscles in MG

The quantitative and descriptive data with respect to EOM outcomes to therapy in juveniles with MG, were sparse, highlighting a research gap (**Table 2**). A large cohort of 306 juveniles with ocular MG from southern China, of whom most were treated with immune therapy in addition to pyridostigmine, only 50% achieved minimal manifestations (43) or better after at least 12 months of follow-up (28). Better outcomes were related to earlier use of "standard treatment" (within 2 years of symptom onset), which included the use of prednisone 0.25 mg/kg/day if symptoms did not resolve with pyridostigmine alone, followed by a slow taper and steroid cessation after 6 months of clinical remission (28). Another large study from China, in which 95% of juveniles had ocular involvement, only 17% "improved" while the remainder were either unchanged or worse, despite immune treatments (advising prednisone 0.75

TABLE 2 | Outcomes of extraocular muscles in juveniles with MG by region.

	Region	AAO, years	N	Follow-up, years (mean)	Ocular outcomes: good vs. treatment resistance as %	OMG patients on immune treatment
Kim et al. (40)	S/Korea	<15	24	3.1	NR; 10% TRO	75%
Lee et al. (7)	S/Korea	<18	88	>2.6	65% vs. 0	>55%
Kraithat et al. (41)	Thailand	<15	14	6.3	93% vs. 7%	79%
Vanikieti et al. (29)	Thailand	<15	62	>4	NR; 8% TRO	52%
Huang et al. (28)	China	<18	306	>1	NR; 50% in remission	93%
Gui et al. (5)	China	<14	424	>5	NR; most unchanged/worse	100%
Ortiz and Borchert (42)	US	<12	21	6.5	NR; OMG resolved in 19%	29%
Xu et al. (9)	US	<18	22	NR	NR; 0 TRO	"Almost all"

AAO, age at onset; OMG, ocular myasthenia gravis; N, refers to sample size; Ocular outcome: "Good" refers to remission or minimal symptoms and "treatment resistance" refers unchanged or worse; TRO refers to treatment-resistant ophthalmoplegia. S/Korea, South Korea; NR refers to not reported.

mg/kg/day with poor responses to pyridostigmine), and even thymectomies (5).

The retrospective results of hospital-based pediatric clinics in South Africa showed, after a median follow-up of 5 years, 31% of prepubertal children ($n = 31$) remained with partial or complete treatment-resistant ophthalmoplegia, and 12% in the postpubertal group ($n = 20$) (11). Although immune treatments were used in this case series, the treatment protocols varied from site to site. In contrast, the pediatric group from North America ($n = 22$; 40% of children with African ancestry) in which >80% were treated within a median of 5 months from symptom onset, and using doses of prednisone 2.5 mg/kg/day for 4–6 weeks before a reduction to alternate day dosing, resulted in all the patients reaching minimal manifestation status or better (9).

In a cohort of predominantly adult MG patients, longitudinal observational data to assess the duration of immune treatment required before the resolution of MG-induced EOM paresis showed that starting immune therapy earlier (<12 months of symptom onset) and using higher doses of prednisone in the first 3 months (0.45 vs. 0.29 mg/kg) associated with significantly better outcomes; patients whose ophthalmoplegia resolved within 3 months of starting therapy had received the higher dose compared to those who only showed resolution of ophthalmoplegia between 4 and 12 months (38). Although there were only nine of 76 patients with MG manifesting with MG before the age of 20 in this cohort, the younger people were less likely to show resolution at 12 months compared to the older people (statistical analyses were not performed due to sample size). Of those with EOM weakness at baseline, 24% remained with complete ophthalmoplegia (all the 12 EOMs with persistent paresis) at 12 months despite moderate doses of prednisone ≈0.35 mg/kg daily with/without steroid-sparing agents (38). These results support the treatment recommendations from Kupersmith and Ying to use earlier and higher doses of prednisone, up to 60 mg daily, for short periods in treating the EOM manifestations of MG (44).

An international working group advising on therapies for juvenile MG recommended starting cholinesterase inhibitors at 0.5 to 1 mg/kg every 4 to 6 h and increasing the dose to 7 mg/kg/day in divided doses for symptom control (3). In our experience, cholinesterase inhibitors may produce some symptomatic relief to the ocular manifestations of MG, especially ptosis, but rarely result in resolution of symptoms; however, others have noted that >50% of patients improve symptomatically on cholinesterase treatment (10). Oral steroids, between 0.5 and 1 mg/kg daily (or 1.5 mg/kg alternate days), are advised in increasing doses in juveniles not responding to cholinesterase inhibitors, with lower doses advised in children with only ocular manifestations (3). Several groups recommend adding steroid-sparing agents to prednisone in children in the setting of poor treatment responses to steroids (3, 5, 11, 12, 28). Steroid-sparing agents which are used in juveniles include azathioprine, mycophenolate mofetil, and rituximab (3). Although methotrexate is increasingly accepted as a cost-effective adjunct to the MG therapeutic armamentarium in adults (45) based on decades of experience in the juvenile arthritides among others, we also use methotrexate in children (10–15 mg/m^2/week plus folic acid >24 h after methotrexate (folate dose≈ 1/3 of methotrexate) (46).

Differential Diagnosis for Treatment-Resistant Seronegative Ocular Myasthenia

Treatment-refractory ophthalmoparesis/plegia among particularly the prepubertal group of juveniles with AChR-Ab negative MG or MuSK-Ab negative MG, may raise the possibility of a congenital myasthenic syndrome (CMS). CMS usually manifests with features of fatigable ocular or generalized muscle weakness at birth or within the first year of life, and often with a family history of a similar phenotype (47). However, pathogenic variations in several CMS genes may manifest in childhood (*CHNRE*; *COLQ*; *DOK7; GFPT1; RAPSN)*, adolescence (*DPAGT1*), or even in adulthood (*CHRNA1*; *CHRNE*; *DOK7; GFPT1; RAPSN*) (47). Most of these CMS are accompanied by additional features such as dysmorphism (*CHRNA1*), or limb-girdle pattern of weakness (*GFPT1*; *GMPPB; DGPAGT1*) without EOM weakness or ptosis. Pathogenic gene variants in a few CMS genes may rarely cause diagnostic confusion with "treatment resistant ocular ± generalized myasthenia"; pathogenic variants in *CHRNE1* have been reported to present after infancy with mild ptosis or

ophthalmoplegia and respond to cholinesterase inhibitors; *DOK7* pathogenic variants may present with limb-girdle weakness and ptosis; occasional pathogenic variants in *RAPSN* may cause fluctuating ptosis with/without generalized fatigability (36, 47). Although pathogenic variants in *COLQ* usually cause severe early onset axial weakness with sparing of EOMs, some cases may have later onset, milder disease with variable occurrence of ophthalmoplegia and ptosis; these patients do not respond to cholinesterase inhibitors (36).

Treatment-Resistant Ophthalmoplegia and Definitions

Myasthenic involvement of the EOMs, similar to non-ocular muscles, is expected to respond to immunosuppressive therapies (38). However, in 2007, we first highlighted the occurrence of chronic treatment-resistant ophthalmoparesis (or ophthalmoplegia) in a subset of patients with MG from South Africa, whereas their non-ocular muscles responded to immune therapies. Treatment-resistant ophthalmoplegia occurred more frequently in those with younger onset (<20 years), AChR-Ab positive MG, and in individuals with African genetic ancestry (48). Subsequently, cross-sectional data from different pediatric centers across South Africa showed that up to 30% of the children attending hospital-based clinics remained with degrees of ophthalmoplegia after several years of immune therapies, irrespective of whether they had ocular-only or generalized MG (11).

Although complete ophthalmoplegia (also referred to as "eyeball fixation") (6) is mentioned in juvenile cohorts from Asia, and elsewhere, it is frequently not quantified. Nevertheless, a Korean cohort of childhood-onset ocular MG (onset before 15 years and follow-up > 6 months) reported that only 29% (of 24 cases) improved in response to treatment with pyridostigmine and prednisone and 10% of patients remained with total ophthalmoplegia; only 50% were treated with prednisone and pyridostigmine (40) (**Table 2**). Treatment-resistant ophthalmoplegia was also reported in cohorts from Italy (3 of 19, 15%) and Canada (1 of 25, 4%) comprising either childhood-onset generalized or ocular MG and was frequently treated with immunosuppressive therapies and thymectomies (13, 49). Children with ocular MG from the USA ($n = 21$; followed for 2 years) showed "limitation of ductions" in 81% and complete resolution of myasthenic signs occurred in only 19%, although only a third had received steroids (42). Treatment resistance requiring oculoplastic surgery was reported in 6% of mainly Caucasian juveniles in another US cohort (19).

Taken together, younger African and Asian children with myasthenic involvement of EOMs appear to be at greater risk of developing treatment-resistant ophthalmoplegia (11, 29, 40). It is important to note that adult-onset MG cases, irrespective of ocular only MG or generalized MG, may develop treatment-resistant ophthalmoplegia including those with MuSK-Ab positive MG, triple seronegative MG, and older men with AChR-Ab positive MG (38, 50–52).

Presently, there is no definition for treatment-resistant or refractory ophthalmoplegia in MG. Definitions related to refractory generalized MG do not apply as patients with ophthalmoplegia (± ptosis) may experience substantial visual disability while the remaining non-ocular muscles may not be severely weak. In addition, refractoriness in generalized disease often requires documentation of treatment non-responsiveness and failure to prevent severe generalized MG weakness or crisis after trying several immune therapies for 12 to 24 months (53, 54), whereas observations suggest EOMs are vulnerable to shorter periods of inactivity due to functional denervation. Therefore, the definition of treatment-resistant ophthalmoplegia cannot be conservative as waiting for long periods in this setting may be counterproductive and contribute to muscle atrophy (**Figure 1**). Longitudinal observations of new patients with MG with persistent ophthalmoparesis/plegia and the timing of their resolution (or not) to immune therapy suggest that a signal for treatment non-responsiveness in most cases is evident around 6–7 months (38). However, another scenario occurs in which patients with MG may only manifest treatment-resistant ophthalmoplegia later, even after initially showing treatment responsiveness of their EOMs; in these cases, usually in the context of generalized disease, we noted that a critical event (infection; abrupt non-compliance) resulted in a relapse of MG and ophthalmoplegia with ongoing persistent non-responsiveness of the EOMs while the non-ocular muscles responded to the re-introduction/adjustments of MG therapies. We postulate that these events may have triggered critical biological pathways (see below) (39).

The clinical examination in patients with MG with chronic treatment-resistant ophthalmoplegia may also vary; some patients show an initial brief quiver movement as the saccadic movement is initiated before the eye stops short of its reduced trajectory, or brief lid twitches with attempted upgaze after a period of downgaze may be observed. However, after years of treatment-resistant ophthalmoplegia, the EOMs of some patients with MG show very limited and slow movements, and in some, there is no observable movement at all. When there is complete ophthalmoplegia, forced duction testing by an ophthalmologist may distinguish whether an apparently "fixed" eyeball can move through its trajectory; this can distinguish between severe eye muscle paralysis, where there is no mechanical restriction to forced EOM duction, and a restrictive force which prevents ocular movement (infiltration or fibrosis). In the setting of concomitant thyroid eye disease, the EOMs would show limited mechanical movement (39).

Genetic Differences of Juvenile MG by Race/Population

Human Leukocyte Antigen Genes

The HLA region on chromosome 6 was the first genetic region, encompassing various class I (*HLA-A, HLA-B*) and class II genes (*HLA-DR, HLA-DQ*), shown to associate with MG (55). These HLA genes encode molecules that present antigens to CD4+ T helper cells which are necessary to mount an adaptive immune response specific to foreign pathogens [reviewed in Nel et al. (56)]. Although many HLA association studies have been performed in adults with MG, those in juveniles and children are sparse, but may suggest that juvenile and/or ocular MG may have

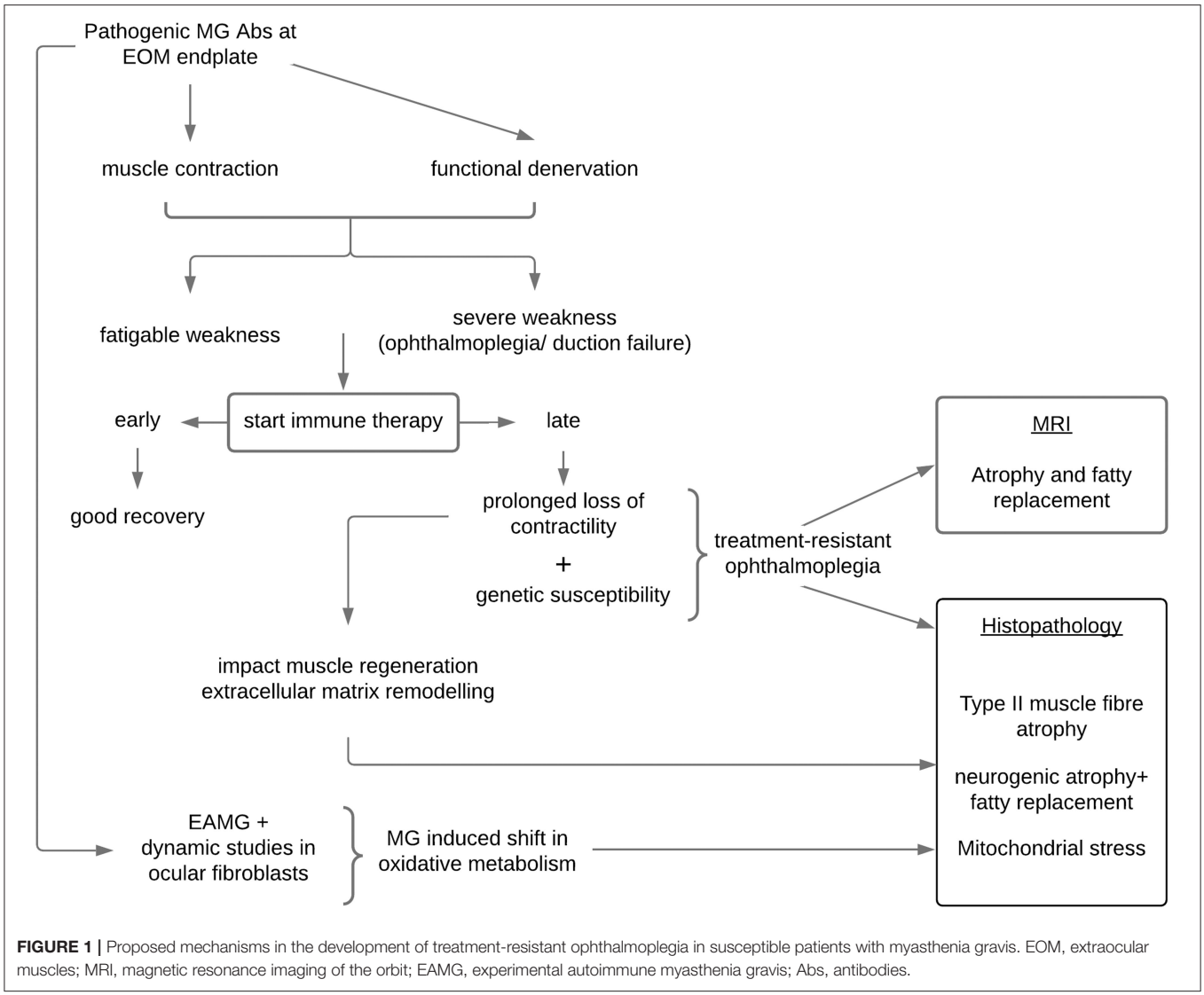


FIGURE 1 | Proposed mechanisms in the development of treatment-resistant ophthalmoplegia in susceptible patients with myasthenia gravis. EOM, extraocular muscles; MRI, magnetic resonance imaging of the orbit; EAMG, experimental autoimmune myasthenia gravis; Abs, antibodies.

a distinct immunological basis in certain populations (**Table 3**). For example, children from Norway showed an association with *DRB1*04* (61) whereas those from Asia were associated with *DRB1*09*. Childhood-onset ocular MG in Japanese and Chinese children, who were predominantly AChR-Ab negative, have shown reasonably consistent *HLA-B*4601; DRB1*0901* associations. Nel et al., found a higher frequency of functional variants in the *HLA-DRB1* region in a selected sample of African juveniles with treatment-resistant ophthalmoplegia (see below) compared to MG cases who responded to therapy (65), as well as the closely linked *HLA-DPB1* region (2). Preliminary results suggest that "low expression" *HLA-DPB1*105:01* genotypes, which were also more common in African controls compared to European controls, associated with African juveniles with treatment-resistant ophthalmoplegia (2).

Pathogenic Mechanisms of Treatment-Resistant Ophthalmoplegia in MG

Our current hypothesis is that in a genetically susceptible individual, treatment-resistant ophthalmoplegia is likely the result of a complex network of dysregulated genes "activated" within the context of MG (39). Against this backdrop and together with a critical period of loss of contractility in the EOMs, muscle atrophy-pathways and mitochondrial metabolic pathways are not able to maintain normal homeostasis, and the paralysis of the EOMs may enter an irreversible phase of mitochondrial stress, EOM atrophy, and fat replacement (66, 67). Importantly, these histopathological changes may not be peculiar to MG, but rather to EOMs (more than other skeletal limb muscles) being particularly vulnerable to atrophy when contractility is compromised for a critical period irrespective of the cause (67). Similar to the EOM histopathological findings, imaging of the EOMs in patients with MG with chronic refractory ocular symptoms, found evidence of muscle atrophy and fatty replacement (52). Interestingly, fatty replacement with larger muscle volume was evident in the EOMs of a pilot case series (feasibility study) earlier in their disease course (68), whereas those with a longer disease duration showed muscle atrophy (69).

Gene expression studies in the EOMs of experimentally induced MG in rodents have also pointed to altered oxidative

TABLE 3 | Human leukocyte antigen (HLA) associations in juvenile myasthenia gravis by racial ancestry or geographical area.

Type	Symptom onset, y	HLA gene associations	Geographical area
Pre-pubertal MG	<10 <12	-DR9; Dw13 -DRB1*0404	Japan (57, 58) China (59, 60) Norway (61)
Post-pubertal MG	12-18	-B*08	Norway (61)
Juvenile MG	<15 <20	-DRB1*0901 -DRB4*0101	China (59) Japan (58, 62)
Ocular MG	<15 <18	-DQA1*0302; DQB1*0303:02	China (63) Japan (62)
		-DRB1*1302; DQA1*0102; DQB1*0604	
		-DRB1*0901; DQA1*0301; DQB1*0303	
		-B*4601; DRB1*0901	China (59, 64)
		-B*4601; DRB1*0403	

*MG to myasthenia gravis. Ocular MG when MG has been confined to ocular muscles for >2 years. Y, years. Both serological and molecular HLA typing methods were considered. HLA alleles derived from molecular typing are denoted with an asterix (*) e.g., DRB1*0901 is the gene for the serotype DR9. For more detail on the curation of HLA studies see Nel and Heckmann (56).*

metabolism (70) which may in turn impact on EOMs maintaining high firing rates and generating contractile force (**Figure 1**). Poor muscle force generation affects mitochondrial biogenesis and triggers muscle atrophy signaling pathways (71, 72) all of which have been shown to be relevant in MG *in-vitro* modeling (73). The patient developing treatment-resistant ophthalmoplegia may be genetically susceptible to the induction of these "dysregulated" pathways only when they develop MG and possibly enter an irreversible stage when not treated early enough.

Although genetic studies have been limited due to the rarity of these patients, candidate gene approaches in juvenile AChR-Ab positive generalized patients with MG with the treatment-resistant ophthalmoplegic phenotype showed associations with regulatory variants in both the *DAF* (*-198 C>G*) and *TGFB1* (*-387 C>T*) genes (74, 75). However, these genetic associations did not account for many of the cases.

An unbiased genome-wide analysis in a highly selected enriched group of juveniles with treatment-resistant ophthalmoplegic MG compared to a matched control group of young myasthenic responders (extreme phenotype approach) identified several genes by their putative functional gene variant burden, which associated with ophthalmoplegic cases (2). Prioritizing these genes by their expression levels in muscle showed they converged on muscle atrophy signaling and myosin II function pathways (2). These predictions were validated in gene expression studies using orbital muscle biopsies of MG cases compared to an independent control group without MG, pointing to dysregulated muscle networks in the ophthalmoplegic MG cases involving muscle atrophy and/or contractility as well as oxidative metabolism gene pathways (76). These pathways identified by gene variant burden, showed significant dysregulated correlations (which differed from controls) with known MG genes/pathways (70, 73), highlighting the importance of the MG context. The unmet need is developing a prognostic biomarker for the early detection of these cases.

CONCLUDING REMARKS

In juveniles with myasthenia, there are phenotypic differences amongst different populations in their ages at presentation, the proportions of ocular vs. generalized manifestation of MG, and in the treatment responsiveness of EOMs to immune therapies. Although ocular MG in younger children is often benign and self-limiting, indications are of genetically susceptible individuals who require a more aggressive approach with immune therapy to avoid chronic visual morbidity. There is a critical need for a prognostic biomarker to guide treatment approaches. In addition, clear knowledge gaps were identified; there is a lack of standardized use of descriptions of eye muscle involvement in juveniles with MG, and poor descriptions of their responsiveness (or lack thereof) to immune therapies. The field will benefit from a collaborative response to these research gaps.

AUTHOR CONTRIBUTIONS

JH conceived the idea and wrote the first draft and prepared **Table 2**. AS performed the literature review and editorial input and prepared **Table 1**. TE and MN provided editorial input and prepared the figure and **Table 3**. All authors contributed to the article and approved the submitted version.

FUNDING

JH and TE received funding from the South African (SA) National Research Funding Agency and MN is the recipient of a CReATe scholars award and a Carnegie Developing Emerging Academic Leaders (DEAL) award. This publication was made possible (in part) by a grant from Carnegie Corporation of New York and a L'Oréal-UNESCO For Women in Science South African Young Talents Award. AS is supported by an educational grant from Life Healthcare.

REFERENCES

1. Soltys J, Gong B, Kaminski HJ, Zhou Y, Kusner LL. Extraocular muscle susceptibility to myasthenia gravis: unique immunological environment? *Ann N Y Acad Sci.* (2008) 1132:220–4. doi: 10.1196/annals.1405.037
2. Nel M, Mulder N, Europa TA, Heckmann JM. Using whole genome sequencing in an african subphenotype of myasthenia

gravis to generate a pathogenetic hypothesis. *Front Genet.* (2019) 10:136. doi: 10.3389/fgene.2019.00136
3. Munot P, Robb SA, Niks EH, Palace J. ENMC workshop study group. 2020. 242nd ENMC International Workshop: Diagnosis and management of juvenile myasthenia gravis Hoofddorp, the Netherlands, 1-3 March 2019. *Neuromuscul Disord.* (2020) 30:254–64. doi: 10.1016/j.nmd.2020.02.001
4. Murai H, Yamashita N, Watanabe M, Nomura Y, Motomura M, Yoshikawa H. Characteristics of myasthenia gravis according to onset-age: Japanese nationwide survey. *J Neurol Sci.* (2011) 305:97–102. doi: 10.1016/j.jns.2011.03.004
5. Gui M, Luo X, Lin J, Li Y, Zhang M, Zhang X, et al. Long-term outcome of 424 childhood-onset myasthenia gravis patients. *J Neurol.* (2015) 262:823–30. doi: 10.1007/s00415-015-7638-2
6. Feng HY, Wang HY, Liu WB, He XT, Huang X, Luo CM, et al. The high frequency and clinical feature of seronegative myasthenia gravis in Southern China. *Neurol Sci.* (2013) 34:919–24. doi: 10.1007/s10072-012-1159-x
7. Lee HN, Kang HC, Lee JS, Kim HD, Shin HY, Kim SM. 'Juvenile Myasthenia Gravis in Korea: Subgroup Analysis According to Sex and Onset Age. *J Child Neurol.* (2016) 31:1561–8. doi: 10.1177/0883073816666206
8. Wang W, Chen YP, Wang ZK, Wei DN, Yin L. A cohort study on myasthenia gravis patients in China. *Neurol Sci.* (2013) 34:1759–64. doi: 10.1007/s10072-013-1329-5
9. Xu L, Castro D, Reisch JS, Iannaccone ST. Response to treatment in pediatric ocular myasthenia gravis. *Muscle Nerve.* (2020) 61:226–30. doi: 10.1002/mus.26745
10. Barraud C, Desguerre I, Barnerias C, Gitiaux C, Boulay C, Chabrol B. Clinical features and evolution of juvenile myasthenia gravis in a French cohort. *Muscle Nerve.* (2018) 57:603–09. doi: 10.1002/mus.25965
11. Heckmann JM, Hansen P, Van Toorn R, Lubbe E, Janse E, van Rensburg JMW, et al. The characteristics of juvenile myasthenia gravis among South Africans. *S Afr Med J.* (2012) 102:532–6. doi: 10.7196/SAMJ.5390
12. VanderPluym J, Vajsar FD, Jacob JK, Mah D, Grenier H. Clinical characteristics of pediatric myasthenia: a surveillance study. *Pediatrics.* (2013) 132:e939–44. doi: 10.1542/peds.2013-0814
13. Evoli A, Batocchi AP, Bartoccioni E, Lino MM, Minisci C, Tonali P. Juvenile myasthenia gravis with prepubertal onset. *Neuromuscul Disord.* (1998) 8:561–7. doi: 10.1016/S0960-8966(98)00077-7
14. Popperud TH, Boldingh MI, Rasmussen M, Kerty E. Juvenile myasthenia gravis in Norway: Clinical characteristics, treatment, long-term outcome in a nationwide population-based cohort. *Eur J Paediatr Neurol.* (2017) 21:707–14. doi: 10.1016/j.ejpn.2017.04.003
15. Jastrzebska A, Jastrzebski M, Ryniewicz B, Kostera-Pruszczyk A. Treatment outcome in juvenile-onset myasthenia gravis. *Muscle Nerve.* (2019) 59:549–54. doi: 10.1002/mus.26445
16. Wong V, Hawkins BR, Yu YL. Myasthenia gravis in Hong Kong Chinese. *Pediatric disease Acta Neurol Scand.* (1992) 86:68–72. doi: 10.1111/j.1600-0404.1992.tb08056.x
17. Chou CC, Su IC, Chou IJ, Lin JJ, Lan SY, Wang YS, et al. Correlation of anti-acetylcholine receptor antibody levels and long-term outcomes of juvenile myasthenia gravis in Taiwan: a case control study. *BMC Neurol.* (2019) 19:170. doi: 10.1186/s12883-019-1397-0
18. Ashraf VV, Taly AB, Veerendrakumar M, Rao S. Myasthenia gravis in children: a longitudinal study. *Acta Neurol Scand.* (2006) 114:119–23. doi: 10.1111/j.1600-0404.2006.00646.x
19. Mansukhani SA, Bothun ED, Diehl NN, Mohney BG. Incidence and ocular features of pediatric myasthenias. *Am J Ophthalmol.* (2019) 200:242–49. doi: 10.1016/j.ajo.2019.01.004
20. Vecchio D, Ramdas S, Munot P, Pitt M, Beeson D, Knight R, et al. Pediatric myasthenia gravis: Prognostic factors for drug free remission. *Neuromuscul Disord.* (2020) 30:120-27. 008. doi: 10.1016/j.nmd.2019.11.008
21. Somnier FE. Increasing incidence of late-onset anti-AChR antibody-seropositive myasthenia gravis. *Neurology.* (2005) 65:928–30. doi: 10.1212/01.wnl.0000176067.32186.a3
22. Lai CH, Tseng HF. Nationwide population-based epidemiological study of myasthenia gravis in Taiwan. *Neuroepidemiology.* (2010) 35:66–71. doi: 10.1159/000311012
23. Matsui N, Nakane S, Nakagawa Y, Kondo K, Mitsui T, Matsumoto T. Increasing incidence of elderly onset patients with myasthenia gravis in a local area of Japan. *J Neurol Neurosurg Psychiatry.* (2009) 80:1168–71. doi: 10.1136/jnnp.2008.152637
24. Mombaur B, Lesosky MR, Liebenberg L, Vreede H, Heckmann JM. Incidence of acetylcholine receptor-antibody-positive myasthenia gravis in South Africa. *Muscle. Nerve.* (2015) 51:533–7. doi: 10.1002/mus.24348
25. Gattellari M, Goumas C, Worthington JM. A national epidemiological study of Myasthenia Gravis in Australia. *Eur J Neurol.* (2012) 19:1413–20. doi: 10.1111/j.1468-1331.2012.03698.x
26. Heckmann JM. Juvenile myasthenia gravis. *Eur J Paediatr Neurol.* (2017) 21:696. doi: 10.1016/j.ejpn.2017.07.011
27. Huang X, Liu WB, Men LN, Feng HY, Li Y, Luo CM, et al. Clinical features of myasthenia gravis in southern China: a retrospective review of 2,154 cases over 22 years. *Neurol Sci.* (2013) 34:911–7. doi: 10.1007/s10072-012-1157-z
28. Huang X, Li Y, Feng H, Chen P, Liu W. Clinical Characteristics of Juvenile Myasthenia Gravis in Southern China. *Front Neurol.* (2018) 9:77. doi: 10.3389/fneur.2018.00077
29. Vanikieti K, Lowwongngam K, Padungkiatsagul T, Visudtibhan A, Poonyathalang A. (2018). Juvenile ocular myasthenia gravis: presentation and outcome of a large cohort. *Pediatr Neurol.* (2018) 87:36–41. doi: 10.1016/j.pediatrneurol.2018.06.007
30. Andrews PI, Massey JM, Howard JF, Sanders DB. Race, sex, puberty influence onset, severity, outcome in juvenile myasthenia gravis. *Neurology.* (1994) 44:1208–14. doi: 10.1212/WNL.44.7.1208
31. Yan C, Zhao R, Song J, Feng X, Xi J, Luo S, et al. Comparison of anti-acetylcholine receptor profiles between Chinese cases of adult- and juvenile-onset myasthenia gravis using cell-based assays. *J Neuroimmunol.* (2020) 349:577403. doi: 10.1016/j.jneuroim.2020.577403
32. Popperud TH, Boldingh MI, Brunborg C, Faiz KW, Heldal AT, Maniaol AH, et al. Juvenile myasthenia gravis in Norway: A nationwide epidemiological study. *Eur J Paediatr Neurol.* (2017) 21:312–7. doi: 10.1016/j.ejpn.2016.09.001
33. Parr JR, Andrew MJ, Finnis M, Beeson D, Vincent A, Jayawant S. How common is childhood myasthenia? The UK incidence and prevalence of autoimmune and congenital myasthenia. *Arch Dis Child.* (2014) 99:539–42. doi: 10.1136/archdischild-2013-304788
34. Chiang LM, Darras BT, Kang PB. Juvenile myasthenia gravis. *Muscle Nerve.* (2009) 39:423–31. doi: 10.1002/mus.21195
35. Niks EH, Kuks JB, Verschuuren JJ. Epidemiology of myasthenia gravis with anti-muscle specific kinase antibodies in The Netherlands. *J Neurol Neurosurg Psychiatry.* (2007) 78:417–8. doi: 10.1136/jnnp.2006.102517
36. Finlayson S, Beeson D, Palace J. Congenital myasthenic syndromes: an update. *Pract Neurol.* (2013) 13:80–91. doi: 10.1136/practneurol-2012-000404
37. Cleary M, Williams GJ, Metcalfe RA. The pattern of extra-ocular muscle involvement in ocular myasthenia. *Strabismus.* (2008) 16:11–8. doi: 10.1080/15569520701830992
38. Europa TA, Nel M, Heckmann JM. Myasthenic ophthalmoparesis: Time To resolution after initiating immune therapies. *Muscle Nerve.* (2018) 58:542–49. doi: 10.1002/mus.26172
39. Heckmann JM, Nel M, A. unique subphenotype of myasthenia gravis. *Ann N Y Acad Sci.* (2018) 1412:14–20. doi: 10.1111/nyas.13471
40. Kim JH, Hwang JM, Hwang YS, Kim KJ, Chae J. Childhood ocular myasthenia gravis. *Ophthalmology.* (2003) 110:1458–62. doi: 10.1016/S0161-6420(03)00460-3
41. Kraithat P, Hansapinyo L, Patikulsila P. Treatment Outcomes and Predictive Factors in Pediatric Ocular Myasthenia Gravis. *J Med Assoc Thai.* (2015) 98:883–8.
42. Ortiz S, Borchert M. Long-term outcomes of pediatric ocular myasthenia gravis. *Ophthalmology.* (2007) 115:1245–48. doi: 10.1016/j.ophtha.2007.10.022
43. Jaretzki RJ, Barohn RM, Ernstoff HJ, Kaminski JC, Keesey AS, Penn DB. Myasthenia gravis: recommendations for clinical research standards. *Task Force Med Scientific Advis Board Myasthenia Gravis Found Am Neurol.* (2000) 55:16–23. doi: 10.1212/WNL.55.1.16

44. Kupersmith MJ, Ying G. Ocular motor dysfunction and ptosis in ocular myasthenia gravis: effects of treatment. *Br J Ophthalmol.* (2005) 89:1330–4. doi: 10.1136/bjo.2004.063404
45. Heckmann JM, Rawoot A, Bateman K, Renison R, Badri M, A. single- blinded trial of methotrexate vs. azathioprine as steroid-sparing agents in generalized myasthenia gravis. *BMC Neurol.* (2011) 11:97. doi: 10.1186/1471-2377-11-97
46. Ferrara G, Mastrangelo G, Barone P, La Torre F, Martino S, Pappagallo G. Methotrexate in juvenile idiopathic arthritis: advice and recommendations from the MARAJIA expert consensus meeting. *Pediatr Rheumatol Online J.* (2018) 16:46. doi: 10.1186/s12969-018-0255-8
47. Finsterer J. Congenital myasthenic syndromes. *Orphanet J Rare Dis.* (2019) 14:57. doi: 10.1186/s13023-019-1025-5
48. Heckmann JM, Owen EP, Little F. Myasthenia gravis in South Africans: racial differences in clinical manifestations. *Neuromuscul Disord.* (2007) 17:929–34. doi: 10.1016/j.nmd.2007.07.002
49. Mullaney P, Vajsar J, Smith R, Buncic JR. The natural history and ophthalmic involvement in childhood myasthenia gravis at the hospital for sick children. *Ophthalmology.* (2000) 107:504–10. doi: 10.1016/S0161-6420(99)00138-4
50. Huda S, Woodhall MR, Vincent A, Heckmann JM. Characteristics Of acetylcholine-receptor-antibody-negative myasthenia gravis in a South African cohort. *Muscle Nerve.* (2016) 54:1023–29. doi: 10.1002/mus.25154
51. Chan JW, Orrison WW. Ocular myasthenia: a rare presentation with MuSK antibody and bilateral extraocular muscle atrophy'. *Br J Ophthalmol.* (2007) 91:842–3. doi: 10.1136/bjo.2006.108498
52. Velonakis G, Papadopoulos VE, Karavasilis E, Filippiadis DK, Zouvelou V, MRI. evidence of extraocular muscle atrophy and fatty replacement in myasthenia gravis. *Neuroradiology.* (2021) 63:1531–8. doi: 10.1007/s00234-021-02753-4
53. Engel-Nitz NM, Boscoe A, Wolbeck R, Johnson J, Silvestri NJ. Burden of illness in patients with treatment refractory myasthenia gravis. *Muscle Nerve.* (2018) 58:99–105. doi: 10.1002/mus.26114
54. Rath J, Brunner I, Tomschik M, Zulehner G, Hilger E, Krenn M, et al. Frequency and clinical features of treatment-refractory myasthenia gravis. *J Neurol.* (2020) 267:1004–11. doi: 10.1007/s00415-019-09667-5
55. Compston DA, Vincent A, Newsom-Davis J, Batchelor JR. Clinical, pathological, HLA antigen and immunological evidence for disease heterogeneity in myasthenia gravis. *Brain.* (1980) 103:579–601. doi: 10.1093/brain/103.3.579
56. Nel, M., and Heckmann, J. M. Epidemiology and genetics of myasthenia gravis. In, *Myasthenia Gravis and Related Disorders* edited Kaminski, H. and Kussner, L. Edition 3 (London: Humana Press). (2018).
57. Matsuki K, Juji T, Tokunaga K, Takamizawa M, Maeda H, Soda M, et al. antigens in Japanese patients with myasthenia gravis. *J Clin Invest.* (1990) 86:392–9. doi: 10.1172/JCI114724
58. Nomura Y, Ehara M, Matsuki K, Juji T, Segawa M, HLA. haplotype in myasthenia gravis (MG) of Japanese children. correlative studies between mg patients and their normal siblings and parents. *Ann N Y Acad Sci.* (1993) 681:581–3. doi: 10.1111/j.1749-6632.1993.tb22947.x
59. Chen WH, Chiu HC, Hseih RP. Association of HLA-Bw46DR9 combination with juvenile myasthenia gravis in Chinese. *J Neurol Neurosurg Psychiatry.* (1993) 56:382–5. doi: 10.1136/jnnp.56.4.382
60. Hawkins BR Yu YL, Wong V, Woo E, Ip MS, Dawkins RL. Possible evidence for a variant of myasthenia gravis based on HLA and acetylcholine receptor antibody in Chinese patients. *Q J Med.* (1989) 70:235–41. doi: 10.1093/oxfordjournals.qjmed.a068318
61. Popperud TH, Viken MK, Kerty E, Lie BA. Juvenile myasthenia gravis in Norway: HLA-DRB1*04:04 is positively associated with prepubertal onset. *PLoS One.* (2017) 12:e0186383. doi: 10.1371/journal.pone.0186383
62. Shinomiya, N., Nomura, Y., and Segawa, M. (2004). A variant of childhood-onset myasthenia gravis: HLA typing and clinical characteristics in Japan. *Clin Immunol* 0.110:154–8. doi: 10.1016/j.clim.2003.10.004
63. Zhu WH, Lu JH, Lin J, Xi JY, Lu J, Luo SS, et al. HLA-DQA1*03:02/DQB1*03:03:02 is strongly associated with susceptibility to childhood-onset ocular myasthenia gravis in Southern Han Chinese. *J Neuroimmunol.* (2012) 247:81–5. doi: 10.1016/j.jneuroim.2012.03.018
64. Feng HY, Yang LX, Liu WB, Huang X, Qiu L, Li Y. The *HLA-B*4601–DRB1*0901 haplotype is positively correlated with juvenile ocular myasthenia gravis in a southern Chinese Han population. *Neurol Sci.* (2015) 36:1135–40. doi: 10.1007/s10072-015-2235-9
65. Nel M, Jalali Sefid Dashti M, Gamieldien J, Heckmann JM. Exome sequencing identifies targets in the treatment-resistant ophthalmoplegic subphenotype of myasthenia gravis. *Neuromuscul Disord.* (2017) 27:816–25. doi: 10.1016/j.nmd.2017.06.009
66. Rautenbach RM, Pillay K, Murray ADN, Heckmann JM. Extraocular muscle findings in myasthenia gravis associated treatment-resistant ophthalmoplegia. *J Neuroophthalmol.* (2017) 37:414–17. doi: 10.1097/WNO.0000000000000534
67. Europa TA, Nel M, Heckmann JM. A review of the histopathological findings in myasthenia gravis: Clues to the pathogenesis of treatment-resistance in extraocular muscles *Neuromuscul Disord.* (2019) 29:381–87. doi: 10.1016/j.nmd.2019.03.009
68. Keene KR, van Vught L, van de Velde NM, Ciggaar IA, Notting IC, Genders SW, et al. The feasibility of quantitative MRI of extra-ocular muscles in myasthenia gravis and Graves' orbitopathy. *NMR Biomed.* (2020) 20:4407. doi: 10.1002/nbm.4407
69. Lueangaram S, Tritanon O, Siriyotha S, Vanikieti K, Padungkiatsagul T, Preechawat P. Radiological characteristics of extraocular muscles in myasthenia gravis patients with ocular manifestations: a case-control study. *Clin Ophthalmol.* (2021) 15:2279–85. doi: 10.2147/OPTH.S280508
70. Kaminski HJ, Himuro K, Alshaikh J, Gong B, Cheng G, Kusner LL. Differential RNA expression profile of skeletal muscle induced by experimental autoimmune myasthenia gravis in rats. *Front Physiol.* (2016) 7:524. doi: 10.3389/fphys.2016.00524
71. Andrade FH, McMullen CA. Lactate is a metabolic substrate that sustains extraocular muscle function. *Pflugers Arch.* (2006) 452:102–8. doi: 10.1007/s00424-005-0010-0
72. Wai Man Y. Extraocular muscles have fundamentally distinct properties that make them selectively vulnerable to certain disorders. *Neuromuscul Disord.* (2005) 15:17–23. doi: 10.1016/j.nmd.2004.10.002
73. Hong Y, Liang X, Gilhus NE. AChR antibodies show a complex interaction with human skeletal muscle cells in a transcriptomic study. *Sci Rep.* (2020) 10:11230. doi: 10.1038/s41598-020-68185-x
74. Heckmann JM, Uwimpuhwe H, Ballo R, Kaur M, Bajic VB, Prince S, et al. functional SNP in the regulatory region of the decay-accelerating factor gene associates with extraocular muscle pareses in myasthenia gravis. *Genes Immun.* (2010) 11:1–10. doi: 10.1038/gene.2009.61
75. Nel M, Buys JM, Rautenbach R, Mowla S, Prince S, Heckmann JM. The African-387 C>T TGFB1 variant is functional and associates with the ophthalmoplegic complication in juvenile myasthenia gravis. *J Hum Genet.* (2016) 61:307–16. doi: 10.1038/jhg.2015.146
76. Europa TA, Nel M, Heckmann JM. *Gene expression profiling of* orbital muscles in treatment-resistant ophthalmoplegic myasthenia gravis. *Orphanet J Rare Dis.* (2020) 15:346. doi: 10.1186/s13023-020-01629-9

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

BRIEF RESEARCH REPORT
published: 28 March 2022
doi: 10.3389/fneur.2022.858998

Analysis of nAChR Autoantibodies Against Extracellular Epitopes in MG Patients

Maria Michail[1,2], *Vasiliki Zouvelou*[3], *Maria Belimezi*[4], *Anna Haroniti*[1], *Marios Zouridakis*[1] *and Paraskevi Zisimopoulou*[1]*

[1] Laboratory of Molecular Neurobiology and Immunology, Hellenic Pasteur Institute, Athens, Greece, [2] Department of Biology, National and Kapodistrian University of Athens, Athens, Greece, [3] Department of Neurology, Eginition Hospital, National and Kapodistrian University of Athens, Athens, Greece, [4] Diagnostic Department, Hellenic Pasteur Institute, Athens, Greece

Myasthenia gravis (MG) is an autoimmune disorder caused by autoantibodies targeting components of the postsynaptic membrane of the neuromuscular junction (NMJ), leading to neuromuscular transmission deficiency. In the vast majority of patients, these autoantibodies target the nicotinic acetylcholine receptor (nAChR), a heteropentameric ion channel anchored to the postsynaptic membrane of the NMJ. Autoantibodies in patients with MG may target all the subunits of the receptor at both their extracellular and intracellular regions. Here, we combine immunoadsorption with a cell-based assay to examine the specificity of the patients' autoantibodies against the extracellular part of the nAChR. Our results reveal that these autoantibodies can be divided into distinct groups, based on their target, with probably different impacts on disease severity. Although our findings are based on a small sample group of patients, they strongly support that additional analysis of the specificity of the autoantibodies of patients with MG could serve as a valuable tool for the clinicians' decision on the treatment strategy to be followed.

Keywords: myasthenia, autoantibodies, anti-nAChR antibodies, cell-based assay, diagnosis of myasthenia, immunoadsorption

OPEN ACCESS

Edited by:
Nils Erik Gilhus,
University of Bergen, Norway

Reviewed by:
Miriam Laura Fichtner,
Yale Medicine, United States
Petra Scholze,
Medical University of Vienna, Austria
Inga Koneczny,
Medical University of Vienna, Austria

***Correspondence:**
Paraskevi Zisimopoulou
zisimopoulou@pasteur.gr

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *20 January 2022*
Accepted: *28 February 2022*
Published: *28 March 2022*

Citation:
Michail M, Zouvelou V, Belimezi M, Haroniti A, Zouridakis M and Zisimopoulou P (2022) Analysis of nAChR Autoantibodies Against Extracellular Epitopes in MG Patients. Front. Neurol. 13:858998. doi: 10.3389/fneur.2022.858998

INTRODUCTION

Myasthenia gravis (MG) is a well-characterized autoimmune disorder caused by autoantibodies (autoAbs) targeting molecules of the neuromuscular junction (NMJ). In MG, the signal transduction caused by the neurotransmitter acetylcholine is impaired and muscle weakness and fatigability occur (1–4).

To date, various MG-specific autoAbs have been identified. One case is the autoAbs against the muscle nicotinic acetylcholine receptor (nAChR) (5), which act according to one of the following three pathogenic mechanisms: (a) activation of the complement at the NMJ, which causes destruction of the typical folds in the sarcolemma, (b) antigenic modulation, which leads to internalization and degradation of the surface nAChR, or (c) blocking of the acetylcholine binding and consequently of the channel opening (6). AutoAbs against the muscle-specific kinase (MuSK) (7) and low-density lipoprotein receptor-related protein 4 (LRP4) (8–10) block the interactions of MuSK and LRP4 and affect the maintenance of the NMJ (6, 11). In addition, other autoAbs with unknown pathogenicity directed against agrin, cortactin, titin, and ryanodine receptor have also been detected in patients' sera with MG (12–16).

The nAChR is a ligand-gated ion channel anchored to the NMJ (17). In humans, two subtypes of the muscle nAChR have been identified, the fetal and the adult subtype. Both the subtypes are heteropentamers composed of 4 subunits forming pentameric assemblies with a stoichiometry of 2α1: β1: ε: δ (adult subtype) or 2α1: β1: γ: δ (fetal subtype) (18–20). Each subunit consists of a ∼210 amino acid extracellular domain (ECD), bearing the epitopes for potential pathogenic autoAbs (21, 22). Although the α1 subunit hosts the main immunogenic region, patients with MG also harbor autoAbs against the non-α1 subunit-ECDs (22–25). AutoAbs against the α1 subunit of the nAChR are characterized as more pathogenic than those against the β1 subunit (26). Furthermore, autoAbs against the γ subunit trigger arthrogryposis in newborns and recognize the fetal subtype of the nAChR on the extraocular muscle in adults (27–30). Thus, the subunit specificity of the anti-nAChR autoAbs seems to influence disease severity.

Currently, the gold standard technique for anti-nAChR autoAbs detection and quantification is a radioimmunoprecipitation assay (RIPA), performed with a mixture of solubilized fetal and adult human nAChR bound to the [^{125}I]-labeled antagonist α-bungarotoxin. RIPA is a reliable technique that provides an accurate estimation of the anti-nAChR autoAbs titer (5, 31). The anti-nAChR autoAbs titer does not correlate with disease severity when patients are compared, although fluctuations in the anti-nAChR autoAbs concentration in an individual patient have been reported to correlate with the severity of muscle weakness and to predict exacerbations. Thus, repeated testing for autoAbs can influence therapeutic decisions (2). Other techniques with good sensitivity and specificity for the detection of the anti-nAChR autoAbs, namely, ELISA, luciferase and fluorescence immunoprecipitation assays, exist. However, these assays have not been widely adopted in clinical practice (2, 32–34). Recently, cell-based assays (CBAs) for the detection of anti-nAChR autoAbs have been developed (35). In brief, CBA utilizes either transiently or stably co-transfected cells with plasmids encoding the five subunits of the nAChR and rapsyn. This co-transfection results in overexpression of the native nAChR on the cell membrane, mimicking the tightly clustered nAChRs on the NMJ. Thus, in addition to other techniques, CBA allows the detection of conformational dependent anti-nAChR autoAbs that recognize discontinuous epitopes and clustered nAChRs (36–41). It has been reported that 16–66% of seronegative patients with MG have autoAbs against the clustered nAChR, detected by CBA (42–46).

Here, we studied the specificity of the anti-nAChR autoAbs in sera, derived from a group of 20 anti-nAChR positive patients with MG at different time points. First, we investigated how many of these patients possess autoAbs against extracellular parts of the nAChR by CBA. We were surprised to find that 7 out of the 20 patients with MG were CBA negative (CBA–), which suggests that they mainly have autoAbs against intracellular parts of the receptor, since these patients were RIPA positive against the native nAChR. Then, only for the CBA positive sera (CBA+), we tested by immunoadsorption the subunit specificity of the autoAbs. Following the immunoadsorption of autoAbs against specific subunit-ECDs, we tested the remaining autoAbs by: (a) RIPA to quantify the percentage of the unbound autoAbs and (b) CBA to test if all the autoAbs against extracellular parts of the nAChR were depleted. Based on our findings, we could divide the tested patients with MG into four groups, according to the target of their autoAbs, which possibly reflects differences in their clinical phenotype.

MATERIALS AND METHODS

Patients

Sera from patients with nAChR-MG, confirmed by RIPA, were provided by the diagnostic department of the Hellenic Pasteur Institute (HPI). The sera samples used were collected from at least two different time points for most of these patients. In total, 55 sera were collected from 20 patients with MG (**Table 1**). Clinical data from 9 patients are available and given in **Table 1**.

Statement of Ethics

The studies involving human participants were reviewed and approved by HPI Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Immobilization of Purified Recombinant Proteins on CNBr-Sepharose Beads

The expressed, in yeast *Pichia pastoris*, ECDs of the human α1, β1, γ, δ, and ε nAChR subunits (47, 48) were immobilized on cyanogen bromide (CNBr)-sepharose beads, after their enzymatic deglycosylation and purification, as described previously (24, 49). In brief, 0.1 mg of ECD and 0.9 mg of bovine serum albumin (BSA) (used as a carrier) were immobilized on 0.1 g of CNBr-activated sepharose beads according to the manufacturer's protocol (GE Healthcare). Following the immobilization, the ECD-carrying beads were diluted in 12 ml phosphate-buffered saline (PBS) containing 0.02% NaN_3. As a control, BSA (1 mg) was immobilized on CNBr-activated sepharose beads.

Immunoadsorption

A total of 125 fmoles of anti-nAChR autoAbs, diluted in PBS/0.2% BSA (total volume: 40 μl), were incubated with 120 μl of sepharose-ECD or sepharose-BSA suspension, for 2 h at room temperature (RT). After centrifugation, supernatants from the immunoadsorption columns containing the unbound anti-nAChR autoAbs were tested by RIPA and CBA.

Radioimmunoprecipitation Assay

For the quantification of the unbound anti-nAChR autoAbs, the autoAb RIPA kit (RSR, UK), containing [^{125}I]-α-bungarotoxin-labeled human fetal and adult muscle nAChR preparations, was used, according to the manufacturer's instructions. From the 160 μl immunoadsorption mix, duplicates of 30 μl samples (containing ∼25 fmoles in case of no depletion) were added to the reaction. The percentage of immunoadsorption was estimated using the equation: 100 × {[ΔcpmBSA] –

TABLE 1 | Results of the tested sera.

	Date	Age	Onset	MGFA	anti-nAChR (nM)	Live CBA	anti-α1 (%)	anti-β1 (%)	anti-γ (%)
Live CBA negative									
P1	2017	n.a.	n.a.	n.a.	820	N	N	N	N
	2021	n.a.	n.a.	n.a.	496	A	N	N	N
P2	2011	n.a.	n.a.	n.a.	275	N	N	N	N
	2013	n.a.	n.a.	n.a.	450	A	N	N	N
	2018	n.a.	n.a.	n.a.	256	A	N	N	N
	2019	n.a.	n.a.	n.a.	480	A	N	N	N
P3	2017	n.a.	n.a.	n.a.	97	A	N	N	N
	2019	n.a.	n.a.	n.a.	39	A	N	N	N
	2020	n.a.	n.a.	n.a.	28	A	N	N	N
P4	2017	n.a.	n.a.	n.a.	65	N			
	2018	n.a.	n.a.	n.a.	46	A			
P5	2011a	28	Early	IIA	272	N			
	2011b	28		IIA	277	N			
	2018	35		PR	130	N			
P6	2010	33	Early	I	341	N			
	2019	42		I	310	N			
P7	1999	51	Early	I	144	N			
	2009	61		I	145	N			
Anti-α1 autoAbs									
P8	2018	79	Late	I	8.8	P	57.84 (±6.07)	N	N
	2019	80		V	36	P	58.38 (±8.83)	N	N
P9	2017	n.a.	n.a.	n.a.	32	P	38.38 (±4.08)	N	N
	2018	n.a.	n.a.	n.a.	36	P	27.55 (±1.82)	N	N
P10	2016	n.a.	n.a.	n.a.	82	P	87.43 (±0.08)	N	N
	2017	n.a.	n.a.	n.a.	40	P	88.06 (±2.16)	N	N
	2020	n.a.	n.a.	n.a.	33	P	88.00 (±1.06)	N	N
P11	2016	n.a.	n.a.	n.a.	16	P	90.70 (±1.65)	N	N
	2017	n.a.	n.a.	n.a.	9	P	93.20 (±1.32)	N	N
	2020	n.a.	n.a.	n.a.	18.7	P	85.47 (±0.31)	N	N
P12	2011	n.a.	n.a.	n.a.	165	P	90.69 (±0.67)	N	N
	2012	n.a.	n.a.	n.a.	77	P	89.61 (±0.12)	N	N
	2019	n.a.	n.a.	n.a.	48	P	92.59 (±0.13)	N	N
Non anti-α1 autoAbs									
P13	2016	73	Late	I	165	P	N	N	65.33 (±2.48)
	2018	75		I	160	P	N	N	67.50 (±3.28)
P14	2016	n.a.	n.a.	n.a.	26	P	N	76.45 (±0.64)	N
	2020a	n.a.	n.a.	n.a.	153	P	N	39.69 (±1.43)	N
	2020b	n.a.	n.a.	n.a.	89	P	N	41.52 (±4.58)	N
	2021	n.a.	n.a.	n.a.	43	P	N	51.90 (±1.82)	N
P15	2013	n.a.	n.a.	n.a.	420	P	N	60.83 (±2.21)	N
	2016	n.a.	n.a.	n.a.	193	P	N	52.08 (±2.33)	N
	2020	n.a.	n.a.	n.a.	208	P	N	57.17 (±1.53)	N
Anti-α1 and non anti-α1 autoAbs									
P16	2017	36	Early	IIA	98	P	27.97 (±3.97)	N	16.15 (±0.83)
	2020	39		IIA	246	P	25.07 (±4.51)	N	15.83 (±7.85)
P17	2007	61	Late	IVB	25.4	P	18.21 (±5.59)	20.01 (±0.73)	34.50 (±4.48)
	2017a	71		IIA	3.5	P	17.51 (±4.32)	14.03 (±3.18)	63.50 (±4.47)
	2017b	71		IIA	2.5	P	16.11 (±1.73)	18.51 (±5.06)	53.51 (±2.13)
	2020	74		IIA	22	P	N	N	38.53 (±5.43)

(Continued)

TABLE 1 | Continued

	Date	Age	Onset	MGFA	anti-nAChR (nM)	Live CBA	anti-α1 (%)	anti-β1 (%)	anti-γ (%)
P18	2011a	n.a.	n.a.	n.a.	65	P	37.37 (±5.16)	N	36.51 (±0.86)
	2011b	n.a.	n.a.	n.a.	14	P	38.38 (±1.54)	N	34.93 (±0.95)
	2017	n.a.	n.a.	n.a.	7.6	P	N	N	50.71 (±0.99)
	2019	n.a.	n.a.	n.a.	12.7	P	59.66 (±1.69)	N	18.26 (±1.36)
P19	2007	48	Early	IVB	8.2	P	56.85 (±6.70)	N	N
	2009	50		IIIB	5	P	N	50.94 (±0.85)	N
P20	2015	34	Early	IVB	11	P	17.51 (±5.96)	N	25.15 (±3.74)
	2018	37		IIB	9.6	P	26.51 (±6.01)	N	35.01 (±7.07)
	2020	39		IIB	198	P	N	44.12 (±4.94)	33.21 (±3.17)

Patients with myasthenia gravis (MG) are grouped by autoAbs specificity. The year of the sample collection, the age of the patients, and the time of disease onset are listed. The distribution and severity of myasthenic weakness were classified according to the MG Foundation of America (MGFA) grading system. The titer of the anti-nAChR autoAbs is given as estimated by RIPA. All the sera were tested for the presence of anti-nAChR autoAbs targeting the extracellular part of the receptor by CBA. The sera were also tested for the presence of the autoAbs against each ECD of the five subunits of the receptor and the percentage of immunoadsorption presented here was estimated as described in "Materials and Methods" section. The average percentage of immunoadsorption from three experiments is presented. In parenthesis, the numbers refer to the ±SD of the immunoadsorption percentage between the different experiments (there was no depletion of autoAbs after the treatment with δ and ε ECD sepharose beads and thus these are not shown in the table).
N, negative; A, ambiguous; P, positive; n.a., not available; PR, pharmacology remission.

[ΔcpmECD]}/[ΔcpmBSA], where Δcpm is the cpm of [^{125}I]-α-bungarotoxin-labeled nAChR (provided in the RSR kit) precipitated by the serum minus that precipitated by a control normal human serum and ΔcpmBSA and ΔcpmECD are the corresponding Δcpm values for samples incubated with immobilized BSA or nAChR-ECD, respectively.

Cell-Based Assay

The CBA was performed as described by Leite et al. (35). Briefly, HEK293T cells were transiently co-transfected with the plasmids encoding for human α1, β1, γ, δ, and ε nAChR subunits and for rapsyn in a ratio of 2:1:1:1:1:1, respectively. Transfection was performed with polyethylenimine (Polyplus). After 48 h, the transfected cells were incubated with serum (20 fmoles of anti-nAChR autoAbs) or supernatant from the immunoadsorption mixture (30 μl containing ∼20 fmoles if no depletion occurred) for 1 h at RT. Afterwards, cells were fixed in 10% formalin solution (Sigma-Aldrich) for 10 min at RT. Patients' anti-nAChR autoAbs were detected after incubation of the cells for 1 h at RT with Alexa Fluor-555 conjugated anti-human IgG Ab (Life Technologies, Invitrogen) in 1:750 dilution. The presence of nAChR on the cell surface was verified by staining with Alexa Fluor-488 labeled α-bungarotoxin (Life Technologies, Invitrogen) in 1:1,000 dilution. Cells were examined under an Olympus IX51 fluorescence microscope by 2 observers.

RESULTS

Detection of AutoAbs Against the Extracellular Parts of the nAChR

We used sera from patients who were tested positive for the presence of anti-nAChR autoAbs at the diagnostic department of the HPI. We chose 20 patients with MG, from whom a recent and at least one previous serum sample were available (55 sera in total). In addition, clinical data for 9 out of the 20 patients were available (**Table 1**). The anti-nAChR autoAbs titer was estimated in all the sera by RIPA (**Table 1**), which detects autoAbs targeting both the extra- and intracellular parts of the nAChR, since solubilized intact nAChRs are used (5, 31). We tested samples containing 20 fmoles of anti-nAChR autoAbs from all sera by CBA. This assay detects only the potential pathogenic autoAbs against adult and fetal subtypes of the nAChR extracellular part (38). We found 18 sera, derived from 7 patients with MG, negative or ambiguous by CBA (**Figure 1** and **Table 1**). This suggests that these patients with MG do not harbor autoAbs targeting extracellular parts of the nAChR or that these autoAbs could not be detected by this method, due to their low concentration in the serum. Interestingly, 3 out of these 7 patients, of whom the clinical data were available, belong to the I-IIA scale according to the MG Foundation of America (MGFA) clinical classification (**Table 1**).

Depletion of AutoAbs Against the Extracellular Domain of the nAChR Subunits

To characterize the autoAbs' subunit-ECD specificity, depletion of autoAbs against the various nAChR ECDs from serum samples was achieved by immunoadsorption (**Figure 1**). For the following experiments, we used immunoadsorption protocols previously established in our laboratory (24, 49). Each immunoadsorption column contained sepharose beads with immobilized either one of the ECDs of the five nAChR subunits (α1, β1, γ, δ, or ε) or only BSA (23, 24, 49). To ensure that all the autoAbs incubated with the immobilized ECDs could be depleted, we used 0.125 pmoles antibodies (Abs)/mg of ECD-sepharose beads, since the capacity of the columns was determined in previous studies to be 1.5 pmoles Abs/mg of immobilized α1-ECD sepharose beads and 5 pmoles Abs/mg of β1-ECD sepharose beads (24, 49).

All the CBA+ MG patients' sera were incubated with the 6 proteins (α1-, β1-, γ-, δ-, ε-ECD, and BSA) immobilized on sepharose beads; unbound autoAbs were then quantified by RIPA and the percentage of immunoadsorption by each column was

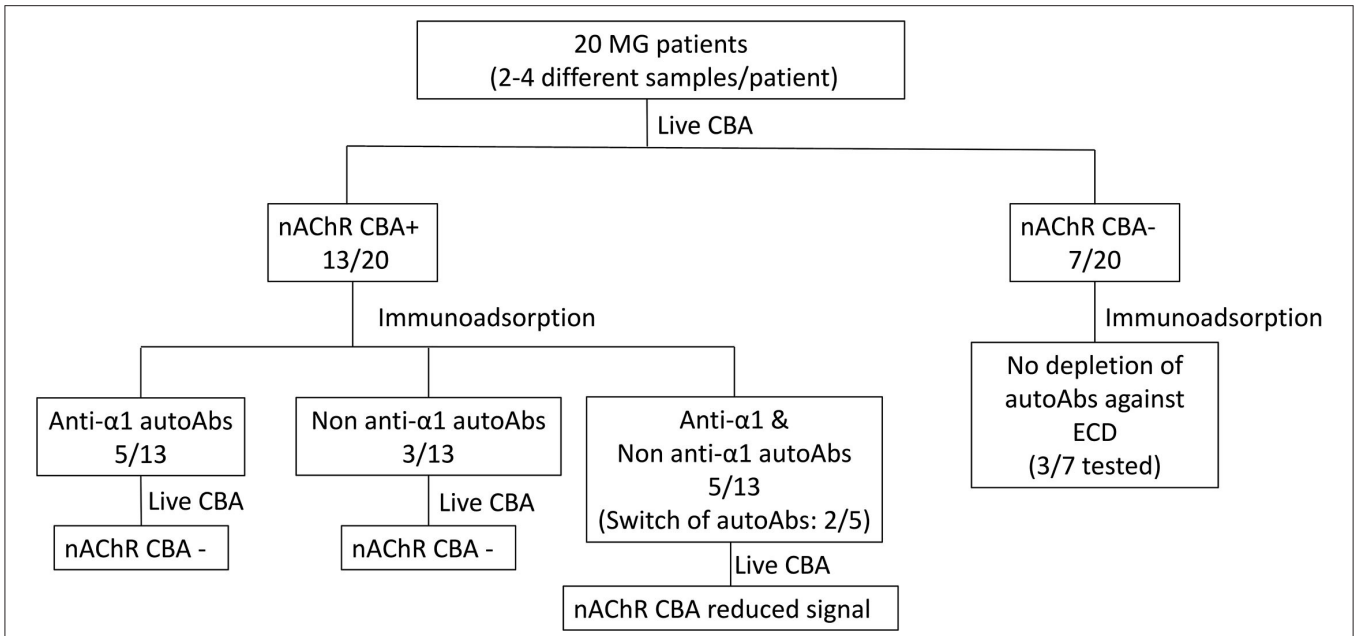


FIGURE 1 | Specificity of the autoAbs derived from patients with myasthenia gravis (MG). Overview of the results.

calculated (**Figure 1** and **Table 1**). From the group of CBA− MG patients, only 3 out of the 7 patients were chosen to be tested by immunoadsorption to verify the absence of any extracellular autoAbs. As expected, there was practically no depletion of autoAbs after incubation with columns containing beads with immobilized nAChR ECDs (**Figure 1** and **Table 1**), confirming the CBA result.

After immunoadsorption, the depleted sera from CBA+ MG patients were further qualified by CBA. More specifically, we investigated if all the autoAbs directed against extracellular epitopes of the nAChR were removed. Based on these experiments, we divided the MG patients tested into the three distinct groups, as given in **Figure 1** and described below:

(a) Patients With MG Harboring autoAbs Against the α1 Subunit.

Five out of 13 CBA+ patients (P8-P12) had anti-α1 autoAbs (**Figure 1** and **Table 1**). After immunoadsorption with the immobilized α1-ECD, these sera were found negative or ambiguous by CBA (**Figures 2A–F**), suggesting that the vast majority of autoAbs targeting extracellular epitopes were depleted by immunoadsorption. Also, data from P8 revealed that an increase of the anti-α1 autoAbs attributed to a higher MGFA score (**Table 1**).

(b) Patients With MG Harboring autoAbs Against the non-α1 Subunits.

Three out of 13 CBA+ patients had non-anti-α1 autoAbs (**Figure 1** and **Table 1**); one patient had anti-γ autoAbs (P13) and two had anti-β1 autoAbs (P14, P15). After immunoadsorption, all sera of the P13 that had been incubated with the γ- immobilized ECD and all sera of the P14 and P15, incubated with the β1-immobilized ECD were negative or ambiguous by CBA. Moreover, P13 who harbors anti-γ autoAbs belongs to the MGFA-I clinical classification, indicating ocular MG (**Table 1**).

(c) Patients With MG Harboring autoAbs Against the α1 and non-α1 Subunits.

Five out of 13 CBA+ patients had anti-α1 and non-anti-α1 autoAbs. More specifically, P16 and P18 had anti-α1 and anti-γ autoAbs, P17 and P20 had anti-α1, anti-β1, and anti-γ autoAbs and P19 had anti-α1 and anti-β1 autoAbs (**Figure 1** and **Table 1**). Interestingly, anti-β1 autoAbs were detected in P19 and P20 for the first time at the second and third samples, respectively, while anti-α1 autoAbs were not detected at those time points (**Figure 1** and **Table 1**). The sera that were treated with the corresponding column when tested by CBA produced a reduced signal (**Figures 2G–O**). In some sera, the reduction of the signal in the CBA was higher than the percentage of immunoadsorption. This is probably due to the depletion of the autoAbs against extracellular epitopes of the receptor after immunoadsorption. The remaining autoAbs were still detected by RIPA but not CBA; this implies that they probably target intracellular epitopes. The clinical data for P16, P17, P19, and P20 indicate that the increase of the non-anti-α1 autoAbs correlates with a decrease in the MGFA score (**Table 1**).

DISCUSSION

Myasthenia gravis is an autoimmune disease caused mainly by autoAbs targeting the nAChR on the NMJ and results in the impairment of neuromuscular transmission and muscle weakness (4). Anti-nAChR autoAbs are heterogeneous and may target all the subunits of the receptor with demonstrated

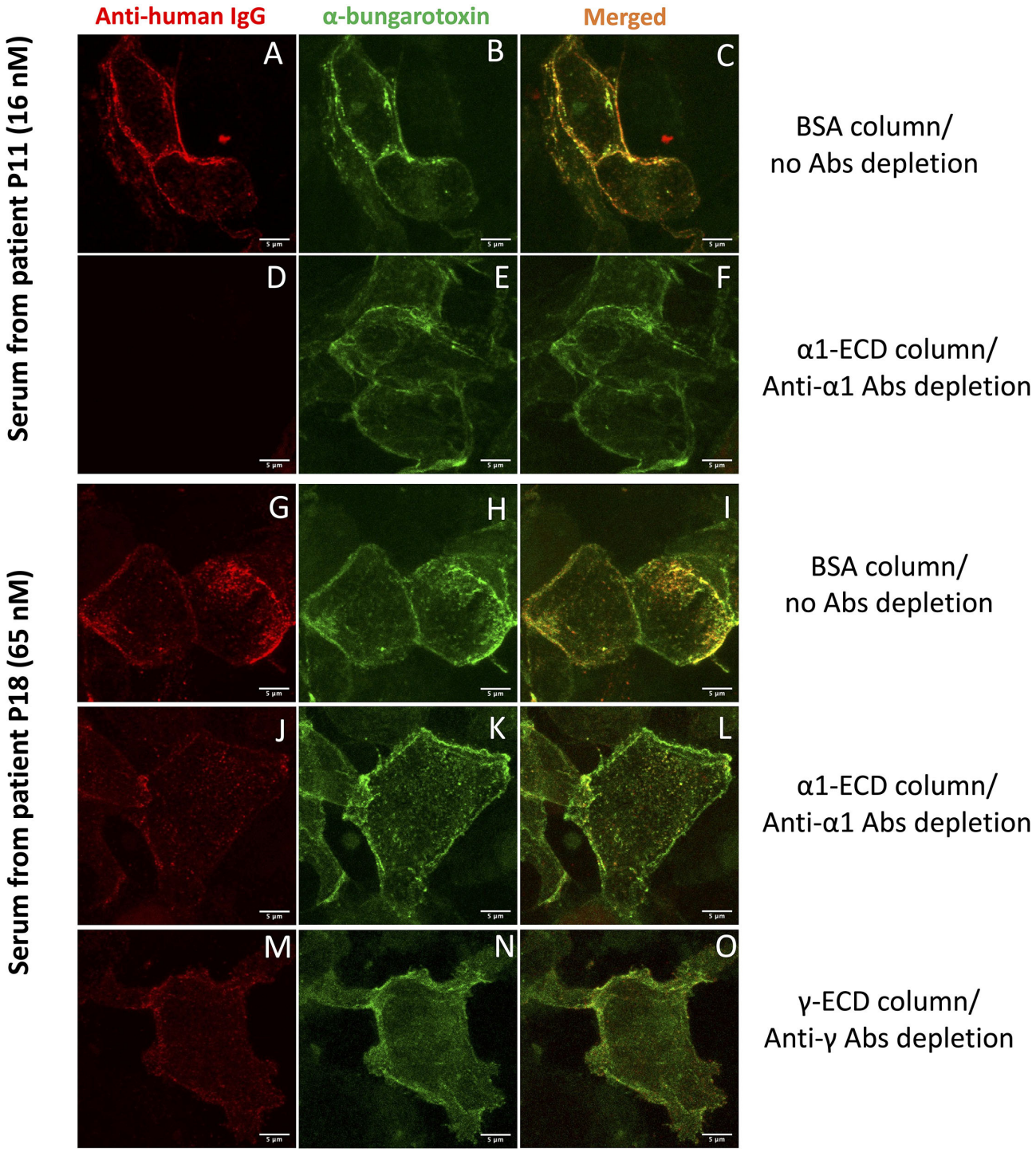


FIGURE 2 | Cell-based assay (CBA) performed with sera after the immunoadsorption assay. HEK293T cells overexpressing a mixture of adult and fetal subtypes of the nAChR were used in the assay. **(A–F)** show cells incubated with serum derived from the patient P11 treated with BSA sepharose beads **(A–C)**, where no autoAbs were depleted or treated with α1-ECD sepharose beads **(D–F)**, where the anti-α1 autoAbs were depleted. **(G–O)** show cells incubated with serum derived from the patient P18 treated with BSA **(G–I)**, α1-ECD **(J–L)**, and γ-ECD **(M–O)** sepharose beads. The first column shows the binding of the specific anti-nAChR autoAbs contained in the serum, visualized by Alexa Fluor-555 labeled anti-human IgG Ab, the second column shows the total number of the nAChR on the cell surface of HEK293T stained with Alexa Fluor-488 labeled α-bungarotoxin. The third column shows merged confocal images of the anti-human IgG and α-bungarotoxin. Images were taken by Leica confocal TCS-SP8 microscope.

different potency for inducing experimental autoimmune MG in rodent models (26, 50, 51). Moreover, epitope spreading against intracellular epitopes, may occur at later stages of MG, as shown in the experimental autoimmune MG rat model, probably due to tissue damage (52).

Here, we studied the specificity of the anti-nAChR autoAbs in sera derived from 20 anti-nAChR positive patients with MG at different time points. We found that 7 out of the 20 patients with MG were negative or ambiguous by CBA (**Figure 1** and **Table 1**). This suggests that the majority of the autoAbs found in these patients' sera is probably against the intracellular parts of the receptor, which are detectable only by RIPA. Interestingly, according to RIPA, these sera had high anti-nAChR autoAbs titer. On the other hand, one could claim that the negative signal of CBA could be attributed to a concentration of autoAbs against extracellular parts of the nAChR well-below the detection limits of the CBA. However, previous studies have also shown patients with MG to be positive by RIPA and negative by CBA (53). The majority of these patients did not have the clinical profile of a neuromuscular transmission disorder, implying that they had no pathogenic autoAbs, which probably recognized intracellular parts of the nAChR (53). Despite the absence of clinical data, we believe that 7 CBA− MG patients in this study fall into the same category as that other study.

Having detected CBA+ patients, we proceeded to further characterization of the autoAbs presented in their sera regarding their subunit specificity. We used immunoadsorption columns appropriate for the depletion of autoAbs targeting the nAChR subunits, as described previously (24, 49). In brief, the columns contained immobilized either the ECD of one of the five subunits of the nAChR (α1, β1, γ, δ, or ε) or BSA. Each serum was incubated with each column and the unbound autoAbs were quantified by RIPA. Moreover, to qualify the unbound autoAbs which recognize extracellular parts of the nAChR, we performed CBA. It is worth mentioning that by testing each serum for the presence of autoAbs against all the five subunits, we also tested the specificity of the bound autoAbs. Based on the results from both techniques, we concluded that CBA+ MG patients tested here can be divided into the three distinct groups: (a) patients with MG harboring anti-α1 autoAbs (5/13), (b) patients with MG harboring non-anti-α1 autoAbs (3/13), and (c) patients with MG harboring anti-α1 and non-anti-α1 autoAbs (5/13) (**Figure 1** and **Table 1**). The CBA signal of all the sera after immunoadsorption was reduced in agreement with the immunoadsorption treatment (**Figures 2A–F**). In fact, in some sera, we observed a higher reduction in the CBA signal (**Figures 2G–O**), compared to the depletion of autoAbs detected in RIPA after immunoadsorption, e.g., although the immunoadsorption percentage of P8's serum after treatment with the immobilized α1-ECD was only 58%, the CBA performed after the immunoadsorption produced no signal. This is probably due to the depletion of the autoAbs against the extracellular epitopes of the receptor after the immunoadsorption. The fact that the remaining autoAbs were detected by RIPA but not by CBA implies that they target intracellular epitopes. In other sera, the CBA signal was negative or ambiguous, suggesting that most autoAbs against the extracellular part of the nAChR were depleted by immunoadsorption. In agreement with previous works, in none of the samples anti-δ or anti-ε autoAbs were detected (23, 24). In general, we observed that the increase of the non-anti-α1 autoAbs correlates with improvement in the disease manifestation (**Table 1**).

The pathogenicity of the anti-α1 autoAbs is well-characterized. The α1 subunit is immunodominant and it can induce experimental autoimmune MG in rats (51). Accordingly, in the anti-α1 autoAbs positive P8 patient, we observed that the increase of the anti-nAChR autoAbs titer correlates with disease deterioration (**Table 1**). The pathogenicity of the anti-β1 autoAbs is less studied and these are thought to be less pathogenic than the anti-α1 autoAbs (26). In fact, in P19 the MGFA score decreased when the autoAbs specificity switched from anti-α1 to anti-β1 autoAbs. Interestingly, in the P20 patient, despite the great increase of the anti-nAChR autoAbs titer, there was no change in the patient's clinical profile upon decrease of the anti-α1 autoAbs and increase of the anti-β1 autoAbs (**Table 1**). Although, the pathogenicity of the anti-γ autoAbs is proved in newborns, in adults they are less pathogenic and may recognize the fetal subtype of the nAChR presented on the extraocular muscle (29, 30, 54). Indeed, P13 who is positive for anti-γ autoAbs has a low MGFA score, which indicates ocular MG (**Table 1**). Moreover, the disease symptoms improve when the anti-γ autoAbs in P17 increase over the anti-α1 autoAbs. Also, there was no difference in the patient's clinical profile when the anti-nAChR autoAbs titer increased, probably due to the presence of only anti-γ autoAbs (**Table 1**). By these observations, we had previously reported a double positive MG patient (anti-nAChR and anti-MuSK autoAbs positive) who was presented with MuSK phenotype (25). This patient's clinical manifestation of the disease was not affected by the increase of the anti-nAChR autoAbs titer. After immunoadsorption, we showed that in all sera from different time points, the patient had relatively small amounts of anti-α1 autoAbs and the vast majority of autoAbs were directed against the β1 and γ subunits. We concluded that the patient did not show any clinical deterioration, because the pathogenic anti-α1 autoAbs were always in low concentration, while the increase of the anti-nAChR autoAbs titer was attributed to the increase of only the less pathogenic anti-β1 and anti-γ autoAbs (25).

Although we do not have a complete clinical profile of all patients, our results support the idea that additional analysis of the autoAbs of patients with MG can provide additional information to the clinicians about the patients' status. This study presents the importance of the CBA technique in the MG diagnosis. It seems that some anti-nAChR positive patients with MG do not harbor pathogenic autoAbs against the extracellular parts of the nAChR or their concentration is under the detection limit of CBA, something that may affect the decision of treatment's strategy. Moreover, we conclude that anti-nAChR positive MG patients can be divided into distinct groups, based on their autoAbs specificity. Consequently, we propose the combination of RIPA and CBA for the follow-up of the MG patients. The former is to be used for the quantification of the

autoAbs and the latter for the identification of the fluctuation of the pathogenic ones.

In future studies, we aim to enlarge our sample group and continue the study of the anti-nAChR autoAbs specificity in MG patients. Moreover, we plan to collect more clinical data from patients with MG and investigate in-depth the correlation of the clinical presentation with autoAbs specificity.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by HPI Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MM conducted the CBAs and immunoadsorption experiments, wrote and edited the manuscript. VZ contributed to the concept of the study, provided the clinical data of the patients, and interpreted the data. MB performed the RIPA experiments. AH conducted confocal microscopy experiments and edited the manuscript. MZ expressed the nAChR ECDs and edited the manuscript. PZ designed the experiments, interpreted the data, and edited the manuscript. All the authors reviewed and approved the final version of the manuscript.

FUNDING

This study was supported by the Stavros Niarchos Foundation (SNF) grant and institutional funding.

REFERENCES

1. Gilhus NE, Verschuuren JJ. Myasthenia gravis: Subgroup classification and therapeutic strategies. *Lancet Neurol.* (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3
2. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis - autoantibody characteristics and their implications for therapy. *Nat Rev Neurol.* (2016) 12:259–68. doi: 10.1038/nrneurol.2016.44
3. Wang S, Breskovska I, Gandhy S, Punga AR, Guptill JT, Kaminski HJ. Advances in autoimmune myasthenia gravis management. *Expert Rev Neurother.* (2018) 18:573–88. doi: 10.1080/14737175.2018.1491310
4. Hughes BW, Moro De Casillas ML, Kaminski HJ. Pathophysiology of myasthenia gravis. *Semin Neurol.* (2004) 24:21–30. doi: 10.1055/s-2004-829585
5. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. *Neurology.* (1976) 26:1054–9. doi: 10.1212/WNL.26.11.1054
6. Rodríguez Cruz PM, Cossins J, Beeson D, Vincent A. The neuromuscular junction in health and disease: molecular mechanisms governing synaptic formation and homeostasis. *Front Mol Neurosci.* (2020) 13:610964. doi: 10.3389/fnmol.2020.610964
7. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med.* (2001) 7:365–8. doi: 10.1038/85520
8. Higuchi O, Hamuro J, Motomura M, Yamanashi Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. *Ann Neurol.* (2011) 69:418–22. doi: 10.1002/ana.22312
9. Zhang B, Tzartos JS, Belimezi M, Ragheb S, Bealmear B, Lewis RA, et al. Autoantibodies to lipoprotein-related protein 4 in patients with double-seronegative myasthenia gravis. *Arch Neurol.* (2012) 69:445–51. doi: 10.1001/archneurol.2011.2393
10. Pevzner A, Schoser B, Peters K, Cosma N-C, Karakatsani A, Schalke B, et al. Anti-LRP4 autoantibodies in AChR- and MuSK-antibody-negative myasthenia gravis. *J Neurol.* (2012) 259:427–35. doi: 10.1007/s00415-011-6194-7
11. Gilhus NE, Tzartos S, Evoli A, Palace J, Burns TM, Verschuuren JJGM. Myasthenia gravis. *Nat Rev Dis Prim.* (2019) 5:50. doi: 10.1038/s41572-019-0079-y
12. Gallardo E, Martínez-Hernández E, Titulaer MJ, Huijbers MG, Martínez MA, Ramos A, et al. Cortactin autoantibodies in myasthenia gravis. *Autoimmun Rev.* (2014) 13:1003–7. doi: 10.1016/j.autrev.2014.08.039
13. Zhang B, Shen C, Bealmear B, Ragheb S, Xiong W-C, Lewis RA, et al. Autoantibodies to agrin in myasthenia gravis patients. *PLoS ONE.* (2014) 9:e91816. doi: 10.1371/journal.pone.0091816
14. Gasperi C, Melms A, Schoser B, Zhang Y, Meltoranta J, Risson V, et al. Anti-agrin autoantibodies in myasthenia gravis. *Neurology.* (2014) 82:1976–83. doi: 10.1212/WNL.0000000000000478
15. Skeie GO, Mygland Å, Treves S, Gilhus NE, Aarli JA, Zorzato F. Ryanodine receptor antibodies in myasthenia gravis: epitope mapping and effect on calcium release *in vitro*. *Muscle Nerve.* (2003) 27:81–9. doi: 10.1002/mus.10294
16. Aarli JA, Stefansson K, Marton LSG, Wollmann RL. Patients with myasthenia gravis and thymoma have in their sera IgG autoantibodies against titin. *Clin Exp Immunol.* (1990) 82:284–8. doi: 10.1111/j.1365-2249.1990.tb05440.x
17. Albuquerque EX, Pereira EFR, Alkondon M, Rogers SW. Mammalian nicotinic acetylcholine receptors: from structure to function. *Physiol Rev.* (2009) 89:73–120. doi: 10.1152/physrev.00015.2008
18. Karlin A. Ion channel structure: emerging structure of the Nicotinic Acetylcholine receptors. *Nat Rev Neurosci.* (2002) 3:102–14. doi: 10.1038/nrn731
19. Le Novère N, Corringer P-J, Changeux J-P. The diversity of subunit composition in nAChRs: evolutionary origins, physiologic and pharmacologic consequences. *J Neurobiol.* (2002) 53:447–56. doi: 10.1002/neu.10153
20. Unwin N. Refined structure of the nicotinic acetylcholine receptor at 4A resolution. *J Mol Biol.* (2005) 346:967–89. doi: 10.1016/j.jmb.2004.12.031
21. Tzartos S, Hochschwender S, Vasquez P, Lindstrom J. Passive transfer of experimental autoimmune myasthenia gravis by monoclonal antibodies to the main immunogenic region of the acetylcholine receptor. *J Neuroimmunol.* (1987) 15:185–94. doi: 10.1016/0165-5728(87)90092-0
22. Tzartos SJ, Lindstrom JM. Monoclonal antibodies used to probe acetylcholine receptor structure: localization of the main immunogenic region and detection of similarities between subunits. *Proc Natl Acad Sci USA.* (1980) 77:755–9. doi: 10.1073/pnas.77.2.755
23. Zisimopoulou P, Lagoumintzis G, Poulas K, Tzartos SJ. Antigen-specific apheresis of human anti-acetylcholine receptor autoantibodies from myasthenia gravis patients' sera using *Escherichia coli*-expressed receptor domains. *J Neuroimmunol.* (2008) 200:133–41. doi: 10.1016/j.jneuroim.2008.06.002
24. Kostelidou K, Trakas N, Tzartos SJ. Extracellular domains of the β, γ and ε subunits of the human acetylcholine receptor as immunoadsorbents for myasthenic autoantibodies: a combination of

immunoadsorbents results in increased efficiency. *J Neuroimmunol.* (2007) 190:44–52. doi: 10.1016/j.jneuroim.2007.07.018
25. Zouvelou V, Michail M, Belimezi M, Zisimopoulou P. Subunit specificity of the acetylcholine receptor antibodies in double seropositive myasthenia gravis. *Muscle Nerve.* (2021) 63:E36–7. doi: 10.1002/mus.27177
26. Kordas G, Lagoumintzis G, Sideris S, Poulas K, Tzartos SJ. Direct proof of the *in vivo* pathogenic role of the AChR autoantibodies from myasthenia gravis patients. *PLoS ONE.* (2014) 9:0108327. doi: 10.1371/journal.pone.0108327
27. Vincent A, Newland C, Beeson D, Riemersma S, Newsom-Davis J, Brueton L, et al. Arthrogryposis multiplex congenita with maternal autoantibodies specific for a fetal antigen. *Lancet.* (1995) 346:24–5. doi: 10.1016/S0140-6736(95)92652-6
28. Kaminski HJ, Kusner LL, Block CH. Expression of acetylcholine receptor isoforms at extraocular muscle endplates. *Invest Ophthalmol Vis Sci.* (1996) 37:345–51.
29. Shi QG, Wang ZH, Ma XW, Zhang DQ, Yang CS, Shi FD, et al. Clinical significance of detection of antibodies to fetal and adult acetylcholine receptors in myasthenia gravis. *Neurosci Bull.* (2012) 28:469–74. doi: 10.1007/s12264-012-1256-0
30. MacLennan C, Beeson D, Buijs AM, Vincent A, Newsom-Davis J. Acetylcholine receptor expression in human extraocular muscles and their susceptibility to myasthenia gravis. *Ann Neurol.* (1997) 41:423–31. doi: 10.1002/ana.410410404
31. Vincent A, Newsom-Davis J. Acetylcholine receptor antibody as a diagnostic test for myasthenia gravis: results in 153 validated cases and 2967 diagnostic assays. *J Neurol Neurosurg Psychiatry.* (1985) 48:1246–52. doi: 10.1136/jnnp.48.12.1246
32. Yang L, Maxwell S, Leite MI, Waters P, Clover L, Fan X, et al. Non-radioactive serological diagnosis of myasthenia gravis and clinical features of patients from Tianjin, China. *J Neurol Sci.* (2011) 301:71–6. doi: 10.1016/j.jns.2010.10.023
33. Hewer R, Matthews I, Chen S, McGrath V, Evans M, Roberts E, et al. A sensitive non-isotopic assay for acetylcholine receptor autoantibodies. *Clin Chim acta.* (2006) 364:159–66. doi: 10.1016/j.cccn.2005.05.035
34. Furukawa S, Akazawa S, Furukawa Y, Kamo I, Satoyoshi E, Hayashi K. A practical enzyme immunoassay for anti-acetylcholine receptor antibodies in myasthenia gravis. *J Neuroimmunol.* (1984) 6:397–409. doi: 10.1016/0165-5728(84)90065-1
35. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, et al. IgG1 antibodies to acetylcholine receptors in "seronegative" myasthenia gravis. *Brain.* (2008) 131:1940–52. doi: 10.1093/brain/awn092
36. Gastaldi M, Scaranzin S, Businaro P, Mobilia E, Benedetti L, Pesce G, et al. Improving laboratory diagnostics in myasthenia gravis. *Expert Rev Mol Diagn.* (2021) 21:579–90. doi: 10.1080/14737159.2021.1927715
37. Rodriguez Cruz PM, Huda S, López-Ruiz P, Vincent A. Use of cell-based assays in myasthenia gravis and other antibody-mediated diseases. *Exp Neurol.* (2015) 270:66–71. doi: 10.1016/j.expneurol.2015.01.011
38. Vincent A, Waters P, Leite MI, Jacobson L, Koneczny I, Cossins J, et al. Antibodies identified by cell-based assays in myasthenia gravis and associated diseases. *Ann N Y Acad Sci.* (2012) 1274:92–8. doi: 10.1111/j.1749-6632.2012.06789.x
39. Rodríguez Cruz PM, Al-Hajjar M, Huda S, Jacobson L, Woodhall M, Jayawant S, et al. Clinical features and diagnostic usefulness of antibodies to clustered acetylcholine receptors in the diagnosis of Seronegative Myasthenia Gravis. *JAMA Neurol.* (2015) 72:642–9. doi: 10.1001/jamaneurol.2015.0203
40. Yan C, Zhao R, Song J, Feng X, Xi J, Luo S, et al. Comparison of anti-acetylcholine receptor profiles between Chinese cases of adult- and juvenile-onset myasthenia gravis using cell-based assays. *J Neuroimmunol.* (2020) 349:577403. doi: 10.1016/j.jneuroim.2020.577403
41. Jacob S, Viegas S, Leite MI, Webster R, Cossins J, Kennett R, et al. Presence and pathogenic relevance of antibodies to clustered acetylcholine receptor in ocular and generalized myasthenia gravis. *Arch Neurol.* (2012) 69:994–1001. doi: 10.1001/archneurol.2012.437
42. Hong Y, Zisimopoulou P, Trakas N, Karagiorgou K, Stergiou C, Skeie GO, et al. Multiple antibody detection in "seronegative" myasthenia gravis patients. *Eur J Neurol.* (2017) 24:844–50. doi: 10.1111/ene.13300
43. Park KH, Waters P, Woodhall M, Lang B, Smith T, Sung J-J, et al. Myasthenia gravis seronegative for acetylcholine receptor antibodies in South Korea: autoantibody profiles and clinical features. *PLoS ONE.* (2018) 13:e0193723. doi: 10.1371/journal.pone.0193723
44. Yan C, Li W, Song J, Feng X, Xi J, Lu J, et al. Cell-based versus enzyme-linked immunosorbent assay for the detection of acetylcholine receptor antibodies in Chinese Juvenile Myasthenia Gravis. *Pediatr Neurol.* (2019) 98:74–9. doi: 10.1016/j.pediatrneurol.2019.01.016
45. Cai Y, Han L, Zhu D, Peng J, Li J, Ding J, et al. A stable cell line expressing clustered AChR: a novel cell-based assay for Anti-AChR antibody detection in Myasthenia Gravis. *Front Immunol.* (2021) 12:666046. doi: 10.3389/fimmu.2021.666046
46. Zhao G, Wang X, Yu X, Zhang X, Guan Y, Jiang J. Clinical application of clustered-AChR for the detection of SNMG. *Sci Rep.* (2015) 5:10193. doi: 10.1038/srep10193
47. Psaridi-Linardaki L, Mamalaki A, Remoundos M, Tzartos SJ. Expression of soluble ligand- and antibody-binding extracellular domain of human muscle acetylcholine receptor alpha subunit in yeast Pichia pastoris. Role of glycosylation in alpha-bungarotoxin binding. *J Biol Chem.* (2002) 277:26980–6. doi: 10.1074/jbc.M110731200
48. Kostelidou K, Trakas N, Zouridakis M, Bitzopoulou K, Sotiriadis A, Gavra I, et al. Expression and characterization of soluble forms of the extracellular domains of the β, γ and ε subunits of the human muscle acetylcholine receptor. *FEBS J.* (2006) 273:3557–68. doi: 10.1111/j.1742-4658.2006.05363.x
49. Psaridi-Linardaki L, Trakas N, Mamalaki A, Tzartos SJ. Specific immunoadsorption of the autoantibodies from myasthenic patients using the extracellular domain of the human muscle acetylcholine receptor α-subunit. Development of an antigen-specific therapeutic strategy. *J Neuroimmunol.* (2005) 159:183–91. doi: 10.1016/j.jneuroim.2004.10.002
50. Tzartos SJ, Bitzopoulou K, Gavra I, Kordas G, Jacobson L, Kostelidou K, et al. Antigen-specific apheresis of pathogenic autoantibodies from myasthenia gravis sera. *Ann N Y Acad Sci.* (2008) 1132:291–9. doi: 10.1196/annals.1405.017
51. Lazaridis K, Baltatzidi V, Trakas N, Koutroumpi E, Karandreas N, Tzartos SJ. Characterization of a reproducible rat EAMG model induced with various human acetylcholine receptor domains. *J Neuroimmunol.* (2017) 303:13–21. doi: 10.1016/j.jneuroim.2016.12.011
52. Feferman T, Im S-H, Fuchs S, Souroujon MC. Breakage of tolerance to hidden cytoplasmic epitopes of the acetylcholine receptor in experimental autoimmune myasthenia gravis. *J Neuroimmunol.* (2003) 140:153–8. doi: 10.1016/S0165-5728(03)00209-1
53. Maddison P, Sadalage G, Ambrose PA, Jacob S, Vincent A. False-positive acetylcholine receptor antibody results in patients without myasthenia gravis. *J Neuroimmunol.* (2019) 332:69–72. doi: 10.1016/j.jneuroim.2019.04.001
54. Dalton P, Clover L, Wallerstein R, Stewart H, Genzel-Boroviczeny O, Dean A, et al. Fetal arthrogryposis and maternal serum antibodies. *Neuromuscul Disord.* (2006) 16:481–91. doi: 10.1016/j.nmd.2006.05.015

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Neurology

ORIGINAL RESEARCH
published: 30 March 2022
doi: 10.3389/fneur.2022.843523

Efficacy and Safety of Tacrolimus Therapy for a Single Chinese Cohort With Very-Late-Onset Myasthenia Gravis

Yiming Zheng[1†], *Xiaoqiu Yuan*[1†], *Caifeng Zhang*[1,2], *Ran Liu*[1], *Haiqiang Jin*[1], *Hongjun Hao*[1], *Fan Li*[1], *Yawen Zhao*[1], *Yun Yuan*[1], *Zhaoxia Wang*[1] *and Feng Gao*[1*]

[1] *Department of Neurology, Peking University First Hospital, Beijing, China,* [2] *North China University of Science and Technology Affiliated Hospital, Qinhuangdao, China*

OPEN ACCESS

Edited by:
Nils Erik Gilhus,
University of Bergen, Norway

Reviewed by:
Sushan Luo,
Fudan University, China
Bitao Bu,
Huazhong University of Science and Technology, China

***Correspondence:**
Feng Gao
gaofh2011@126.com

[†] *These authors have contributed equally to this work and share first authorship*

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *26 December 2021*
Accepted: *15 February 2022*
Published: *30 March 2022*

Citation:
Zheng Y, Yuan X, Zhang C, Liu R, Jin H, Hao H, Li F, Zhao Y, Yuan Y, Wang Z and Gao F (2022) Efficacy and Safety of Tacrolimus Therapy for a Single Chinese Cohort With Very-Late-Onset Myasthenia Gravis. Front. Neurol. 13:843523. doi: 10.3389/fneur.2022.843523

Background and Purpose: Previous studies have found tacrolimus to be a favorable drug for treating different types of myasthenia gravis (MG), but few have focused on very-late-onset MG (VLOMG). This study evaluated the efficacy and safety of tacrolimus for VLOMG therapy.

Methods: This was a retrospective single-center cohort study of 70 patients with VLOMG (onset ≥65 years) who visited Peking University First Hospital in 2019. Participants were divided into the tacrolimus (Tac) group and the control group based on tacrolimus usage. We further divided the Tac group into patients treated without corticosteroids and with corticosteroids. Sociodemographic features, clinical profiles, and outcomes were compared between different therapies and further analyzed by multivariate regression. Details of tacrolimus treatment, comorbidities, and adverse drug reactions (ADRs) were described.

Results: Among 70 patients, the median (interquartile range) age at onset was 71 (68–77) years, and the follow-up duration was 27 (27-29) months. Most patients were types I (28%) and III (40%) according to the MG Foundation of America (MGFA) classification. In the Tac group, tacrolimus treatment was maintained for 36 (27-38) months. The dosage at the final evaluation was 1.0 (1.0–1.75) mg/day, and the last blood concentration test was 4.25 (2.85–5.7) ng/ml. A total of 43% reached remission, and 37% improved based on MGFA postintervention status (MGFA-PIS). For the 9 patients, newly diagnosed at enrollment within this group, MG activities of daily living (MG-ADL) decreased significantly from 3 (2-5) to 2 (1-2) ($p = 0.041$). Regarding the 13 patients, coadministering Wuzhi capsules the tacrolimus concentration increased from 2.75 (1.4–3.8) ng/ml to 5.95 (5.1–7.0) ng/ml ($p = 0.012$). No significant differences in outcomes were observed between tacrolimus treatment without and with corticosteroids or between the Tac group and the control group. A total of 93% had at least one comorbidity. ADRs related to tacrolimus emerged in 25% (9/36) of patients, most of which were not serious and reversible.

Conclusions: Tacrolimus is effective and safe in treating VLOMG. Tacrolimus monotherapy without corticosteroids can be used as an initial and maintenance treatment for VLOMG. Wuzhi capsules work well in elevating tacrolimus concentrations in this population.

Keywords: myasthenia gravis, tacrolimus, late-onset, elderly, Wuzhi capsules, clinical efficacy

INTRODUCTION

Myasthenia gravis (MG) is a neuromuscular junction disorder caused by autoantibodies against acetylcholine receptors (AChR) on the postsynaptic membrane or their adjacent proteins. Patients typically suffer from fluctuating fatigability of skeletal muscles, usually starting with ptosis or diplopia, and in severe cases progressing to respiratory muscle paralysis (1, 2). The recent studies suggested dividing the MG population into three groups based on the onset age using cutoffs of 50 and 65 years. Very-late-onset MG (VLOMG) refers to individuals developing symptoms at ≥ 65 years (3–5), which accounts for the largest proportion of all the MG age groups at present and exhibits an increasing incidence over recent years (3, 6, 7). VLOMG features vs. younger patients of more severe onset, higher risk of exacerbation, fewer medication requirements, better long-term outcomes, and more comorbidities have been reported, indicating potential divergence in treatment selections (3, 5, 8). In addition, age-related dysregulation of the immune system and comorbidities entangle considerations for management. The current treatments for VLOMG mainly work on enhancing hindered transmission (cholinesterase inhibitors) and suppressing overactivated humoral immunity (corticosteroids, immunosuppressors, or monoclonal antibodies). Rapid-acting treatments used in crisis include plasmapheresis and intravenous immunoglobulin (2, 9). Surgical removal of the thymus is less considered for the elderly regarding their comparatively fragile physical conditions and intolerance to surgery (1).

Tacrolimus could be a promising immunosuppressor in managing VLOMG owing to its rapid and long-term efficacy with relatively safe profiles, especially slight nephrotoxicity, which prominently benefits elderly patients (9–12). This agent inhibits T-cell activation by suppressing calcineurin and additionally strengthens muscle contractility by improving ryanodine receptor function (13). Several studies have confirmed the steroid-sparing and symptom-improving effects of tacrolimus in different types of MG (13–16). It is also necessary to clarify medication details for VLOMG, but such research is still lacking. Currently, the auxiliary roles of Chinese medicines in treating autoimmune diseases and preventing complications after transplantation are rising. Notably, Wuzhi capsules (WZC), one preparation of the ethanol extract of Wuweizi or *Schisandra sphenanthera*, have been reported to strongly improve the tacrolimus concentration (17–19). However, no study has focused on WZC usage in the older MG patients. Therefore, we investigated the efficacy and safety of tacrolimus in treating VLOMG and coadministration of WZC in this population and tried to perceive patients' feelings during treatment using patient-reported outcomes and scales of life quality in real-world settings.

Abbreviations: MG, myasthenia gravis; VLOMG, very-late-onset MG; Tac, tacrolimus; ADRs, adverse drug reactions; MGFA, Myasthenia Gravis Foundation of America; MGFA-PIS, MGFA post-intervention status; MG-ADL, MG activities of daily living; AChR, acetylcholine receptor; WZC, Wuzhi capsules; IVIg, intravenous immunoglobulin; MG-QOL-15R, revised 15-item Myasthenia Gravis Quality of Life scale; SSQ, simple single question; EQ-5D-5L, five-level EuroQol five-dimensional questionnaire; IQR, interquartile range; RR, rate ratio; 95%CI, 95% confidence intervals.

MATERIALS AND METHODS

Design and Patients

This study was approved by the Institutional Review Board and Ethics Committee at Peking University First Hospital and was conducted following the ethical standards of the Helsinki Declaration. Informed consent was obtained from each patient included.

The retrospective single-center cohort study was performed under real-world settings in patients with very-late-onset MG (age at onset ≥ 65 years old). Participants included all individuals who visited our institution between January 15, 2019 and December 26, 2019, had a confirmed diagnosis of MG, and first developed symptoms at or after 65 years of age. Diagnoses of MG were made following the guidelines of the Association of British Neurologists based on the typical manifestations of fluctuating muscle weakness and diagnostic test results of antibody positivity or neurophysiological examination positivity (20). Those without contact information or those who refused to participate were excluded. Patients were divided into two groups as follows: the Tac group, participants who had been treated with tacrolimus longer than 3 months [tacrolimus took effect after 3 months of continuous medication in most patients according to previous studies (12, 21)]. Control group, those who had never used tacrolimus or those treated with this drug for less than 3 months. The Tac group was further divided into the non-corticosteroids (those who had never taken corticosteroids) and the corticosteroids group (those who had been treated by corticosteroids). Lost contact was defined as patients who did not answer the phone at least 3 times in different periods of the day or those who left the wrong numbers or empty numbers in records. The flowchart of patient selection in the study is presented in **Figure 1**.

Covariates

Sociodemographic features (sex, age at MG onset, age at the last follow-up, resident area, nationality, marital status,

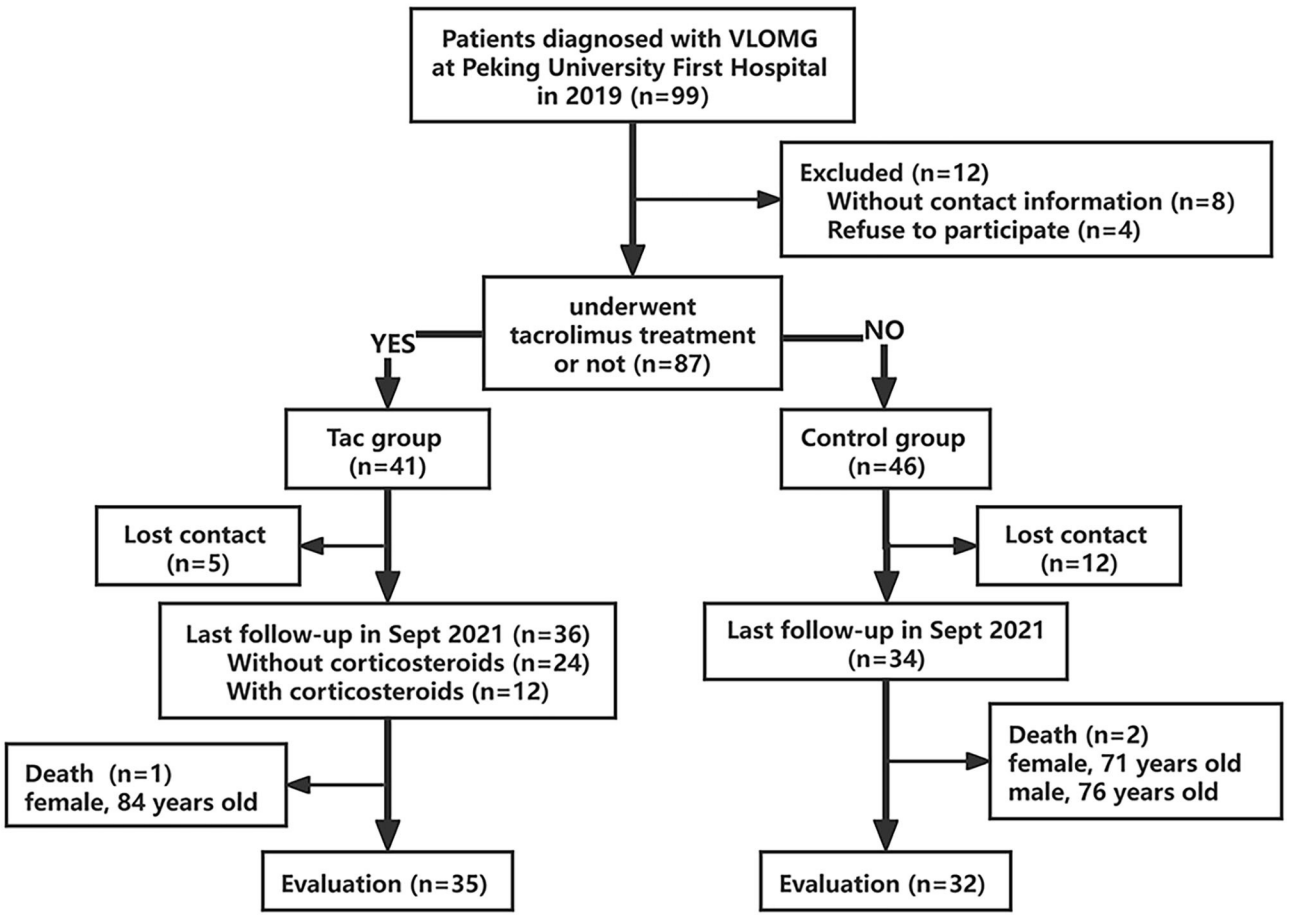


FIGURE 1 | Flowchart of the participants included in this study. VLOMG, very-late-onset myasthenia gravis.

occupation, and educational attainment) were obtained from retrospective analyses of the hospital records. Details of treatments from the patients' medical records and their report at the last follow-up included tacrolimus, cholinesterase inhibitors, corticosteroids, WZC, other immunosuppressors (including azathioprine, cyclosporin A, mycophenolate mofetil, methotrexate, cyclophosphamide, and rituximab), other Chinese medicines (except for the natural product and extracts of Wuweizi or *Schisandra sphenanthera*), concomitant drugs (drugs taken to treat concomitant diseases), intravenous immunoglobulin (IVIg), and plasmapheresis. Additional data on tacrolimus and WZC were collected: age of starting tacrolimus, the dose of tacrolimus at the final evaluation (for those with discontinuation, we collected the dosage before withdrawal documented in the medical records), last tacrolimus concentration, duration of continuous medication (from starting tacrolimus to the last follow-up or discontinuation), WZC usage, and tacrolimus concentration before and after taking WZC.

Information on comorbidities was obtained from medical records, digital questionnaires, and the follow-up telephone interviews as a supplement. This part of the interviews included several closed questions and one open question allowing respondents to add information. Comorbidities being queried included hypertension, coronary diseases, arrhythmia, valvular diseases, peripheral vascular diseases, cerebrovascular diseases, other neurological diseases, diabetes, thyroid diseases, dyslipidemia, hyperuricemia, chronic lung disease, digestive diseases, hepatobiliary disease, kidney diseases, eye diseases, skin diseases, osteoporosis or fracture, depression, infections, hematopoietic diseases, benign prostatic hyperplasia, gynecological diseases, and malignant solid tumor. Here, we used the number of comorbidities to quantify the burden of concomitant diseases. Other collected clinical characteristics included the first visit or revisit at enrollment, inpatient or outpatient visit, length of follow-up, anti-AChR antibody positivity, thymic pathology, MG subtype according to MG Foundation of America (MGFA) classification, and baseline MG activities of daily living (MG-ADL). Anti-AChR antibodies were measured by radioimmunoassay in the neuroimmunology laboratory of Peking University First Hospital. Thymic hyperplasia or thymoma was diagnosed based on the CT

TABLE 1 | Demographical and clinical characteristics of VLOMG patients in the whole cohort and first visit subgroup.

Variables	Whole ($n = 70$)				First visit ($n = 19$)			
	Control group ($n = 34$)	Tac group ($n = 36$)	Total ($n = 70$)	*P* value[a]	Control group ($n = 10$)	Tac group ($n = 9$)	Total ($n = 19$)	*P* value
Demographic characteristics								
Sex				0.084				0.876
Male	23 (68%)	17 (47%)	40 (57%)		7 (70%)	6 (67%)	13 (68%)	
Female	11 (32%)	19 (53%)	30 (43%)		3 (30%)	3 (33%)	6 (32%)	
Age at onset, median (IQR), y	70.5 (67–77)	72 (68.5–78)	71 (68–77)	0.491	70 (68–79)	73 (70–76)	70 (68–78)	0.742
Inpatient at enrollment	10 (30%)	5 (14%)	15 (21%)	0.114	5 (50%)	2 (22%)	7 (37%)	0.210
Follow-up time, median (IQR), m	27 (26–29)	28 (27–29)	27 (27–29)	0.256	26.5 (23–29)	28 (25–29)	27 (25–29)	0.711
Clinical profiles								
Anti-AChR antibody positive	22/31 (71%)[b]	26/31 (84%)	48/62 (79%)	0.224	7 (70%)	7/8 (88%)	14/18 (78%)	0.375
Thymoma or thymic hyperplasia	6 (18%)	10 (28%)	16 (23%)	0.313	1 (10%)	3 (33%)	4 (21%)	0.213
MGFA type				0.020*				0.073
I	15 (44%)	5 (14%)	20 (28%)		6 (60%)	2 (22%)	8 (42%)	
IIa/IIb	5 (15%)	9 (25%)	14 (20%)		0 (0%)	4 (44%)	4 (21%)	
IIIa/IIIb	9 (26%)	19 (53%)	28 (40%)		3 (30%)	3 (33%)	6 (32%)	
IVa/IVb	3 (9%)	3 (8%)	6 (9%)		0 (0%)	0 (0%)	0 (0%)	
V	2 (6%)	0 (0%)	2 (3%)		1 (10%)	0 (0%)	1 (5%)	
MG-ADL at enrollment, median (IQR)	2.5 (0–4)	2 (0–5)	2 (0–4)	0.809	3 (1–3)	3 (2–5)	3 (2–3)	0.365
Details of treatments								
Corticosteroids				0.015*				0.364
Never use	16 (47%)	24 (67%)	40 (57%)		7 (70%)	8 (89%)	15 (79%)	
Using	14 (41%)	4 (11%)	18 (26%)		2 (20%)	0 (0%)	2 (11%)	
Discontinuation	4 (12%)	8 (22%)	12 (17%)		1 (10%)	1 (11%)	2 (11%)	
Other immunosuppressors[c]				0.038*				0.073
Never use	23 (68%)	33 (92%)	56 (80%)		7 (70%)	9 (100%)	16 (84%)	
Using	9 (26%)	2 (6%)	11 (16%)		3 (30%)	0 (0%)	3 (16%)	
Discontinuation	2 (6%)	1 (3%)	3 (4%)		0 (0%)	0 (0%)	0 (0%)	
Cholinesterase inhibitors				0.488				0.161
Never use	9 (26%)	8 (22%)	17 (24%)		2 (20%)	0 (0%)	2 (11%)	
Using	18 (53%)	16 (44%)	34 (49%)		5 (50%)	8 (89%)	13 (68%)	
Discontinuation	7 (21%)	12 (33%)	19 (27%)		3 (30%)	1 (11%)	4 (21%)	
IVIg	8 (24%)	10 (28%)	18 (26%)	0.684	2 (20%)	2 (22%)	4 (21%)	0.906
Plasmapheresis	3 (9%)	0 (0%)	3 (4%)	0.069	0 (0%)	0 (0%)	0 (0%)	——
Other Chinese medicines[d]	9 (26%)	9 (25%)	18 (26%)	0.888	1 (10%)	1 (11%)	2 (11%)	0.937
Concomitant drug(s)[e]	27 (80%)	27 (75%)	54 (77%)	0.660	8 (80%)	8 (89%)	16 (84%)	0.596
Comorbidities[f]	**($n = 32$)**	**($n = 35$)**	**($n = 67$)**		**($n = 9$)**	**($n = 9$)**	**($n = 18$)**	
Without comorbidity	3 (9%)	2 (6%)	5 (7%)	0.569	0 (0%)	1 (11%)	1 (6%)	0.303
Number of comorbidities, median (IQR)	3 (2–5.5)	2 (2–4)	3 (2–4)	0.178	4 (2–5)	2 (1–3)	3 (2–5)	0.194

**Covariates with a $p < 0.05$ were included in multiple regression analysis.*

[a]Compared the Tac group with the Control group.

[b]Incomplete data were described by n/total (%).

[c]Included 8 methotrexate, 3 mycophenolate mofetil, 3 rituximab, 2 azathioprine, 1 cyclophosphamide, and 1 cyclosporin A.

[d]Except for the natural product and extracts of Wuweizi or Schisandra sphenanthera.

[e]Drugs are taken to treat concomitant diseases.

[f]Information on comorbidities was not available for the three deceased patients.

VLOMG, very-late-onset myasthenia gravis; Whole, 67 patients completing the last follow-up except 3 deceased; First visit, 19 patients who first visited our institution and were newly diagnosed with MG at enrollment; IQR, interquartile range; AChR, acetylcholine receptor; MG, Myasthenia Gravis; MGFA, Myasthenia Gravis Foundation of America; IVIg, intravenous immunoglobulin.

scan results. MGFA type and MG-ADL were assessed by the attending neurologists.

Therapeutic Regimens

Treatment was tailored to the individual patient following guidelines, the experience of the clinicians, and patient wishes. For mild patients with ocular MG, cholinesterase inhibitors were prescribed; a single 60 mg of cholinesterase inhibitors (pyridostigmine bromide) was administered when symptoms occurred (≦480 mg/day). Immunosuppression was added when inadequate response occurred or in patients with generalized MG. Prednisone or equivalent methylprednisolone was started at 20 mg/day and increased by 10 mg every 5 to 7 days to the daily target of 0.5–1.0 mg/kg (≦100 mg/day); after 6–8 weeks of maintenance, the dose of steroids was gradually reduced to the lowest effective dose. For those who did not require rapid improvement (such as myasthenic crisis or life being severely affected) or who could not tolerate the side effects of steroids, tacrolimus, and other immunosuppressors were considered. In the Tac group, tacrolimus was administered twice a day at an initial dose of 1.0 mg/day, following the physicians' advice. Dosage was adjusted based on the individual's disease condition, tacrolimus concentration, tests of hematology, and biochemistry. For those who could not achieve adequate concentration (4.8–9.0 ng/ml) (22, 23) by adjusting doses, two tablets of WZC were taken with tacrolimus every time. In the control group, stable doses of other immunosuppressors were as follows: azathioprine 2–3 mg/kg/day, cyclosporin A 2–4 mg/kg/day, mycophenolate mofetil 1.0–1.5 g/day, methotrexate 20 mg/week (with folic acid 1 mg/day), and cyclophosphamide 100 mg/day. Rituximab was administered 375–750 mg/m^2 once every 2 weeks. IVIg (400 mg/kg/day for 5 days) or plasmapheresis (each exchange of 1.0–1.5 plasma volumes and 3–6 times within 10–14 days) were performed in life-threatening cases. Other Chinese medicines and concomitant drugs were applied following doctors of other specialties.

Outcome Measurements and Follow-Up

To evaluate the efficacy of tacrolimus and its impacts on quality-of-life, MGFA postintervention status (MGFA-PIS), MG-ADL scale, relapse (s) or aggravation (s), revised 15-item MG Quality-of-Life scale (MG-QOL-15R), simple single question (SSQ), and five-level EuroQol five-dimensional questionnaire (EQ-5D-5L) were assessed based on semistructured telephone interviews at the last follow-up in September 2021. If patients were not able to answer the questions, close family members were interviewed instead. The primary outcome was MGFA-PIS, the secondary outcome consisting of the other measurements mentioned above. Details of adverse drug reactions (ADRs) were extracted from the interviews.

The MGFA-PIS served as a four-categorical variable in this study: remission (chronic stable remission or minimal manifestations or pharmacological remission), improved, unchanged, and worse or exacerbation (24). In the Tac group, patients were additionally divided into the effective and ineffective groups according to whether MGFA-PIS remission/improvement was achieved. A higher MG-ADL score (range: 0–24) indicates greater MG-related disability (24). The change of MG-ADL was calculated by subtracting the baseline score from the follow-up score. Relapse(s) or aggravation(s) was evaluated by the patients' description after being asked "Have you ever relapsed or aggravated since (the date at enrollment)". Relapse was defined as the reappearance of any symptoms that lasted more than 24 h, and aggravation was defined according to MGFA-PIS criteria of worse or exacerbation. A higher MG-QOL-15R score (range: 0–30) reflects worse physical and psychological functionality (25). The eighth question focusing on work skills might not fit in our patients since the majority were retired. We jumped over it and used 0 as the unified score for this question. The SSQ is a simple and validated question of "What percentage of normal do you feel regarding your MG" (26). Most patients could only name a range, so substitutions (1–10% = 1, 11–20% = 2, and so on) were adopted. EQ-5D-5L health utility values (hereafter referred to as health value) were calculated according to an 8-parameter multiplicative model that performed well in the Chinese individuals (27).

Statistical Analyses

Analyses were performed using Stata version 12.0 (StataCorp LLC). $P < 0.05$ (2-sided) was considered statistically significant. Continuous variables were expressed as medians and interquartile ranges (IQRs) according to a skewed distribution, while categorical variables were described by percentages. Information on patients who lost contact [20% (17/87)] was not collected. Comorbidities, outcome measurements, and ADRs of the deceased [4% (3/70)] were not available. Items with incomplete data (sociodemographic characteristics and anti-AChR antibody positivity) are presented as *n*/total (%). All these missing data were not involved in the statistical analyses.

Confounding factor adjustment was conducted on covariates with $p < 0.05$ in univariable comparisons using multiple linear regression (continuous outcomes) and multiple modified Poisson regression (categorical outcomes). Modified Poisson regression (Poisson regression with robust estimation of variance) instead of logistic regression was applied in this study to allow a more accurate estimation of rate ratio (RR) when the rare event assumption was violated (28).

To evaluate the efficacy of tacrolimus, univariable comparisons of covariates and outcomes were conducted between the Tac group and the control group using the Mann–Whitney U test for continuous variables and the chi-squared test for categorical variables. Covariates with $p < 0.05$ were then included in multivariate regression models. The presence of multicollinearity was evaluated by variance inflation factor estimates. Similar analyses were performed in two subgroups: "First visit," 19 patients who first visited our clinic and were newly diagnosed with MG at enrollment (Tac: 9, control: 10). "MGFA>1," 50 patients whose MGFA type was higher than I (Tac: 31, control: 19). To evaluate the efficacy of tacrolimus monotherapy, univariable comparisons using the Fisher exact test for categorical variables and Mann-Whitney U test for

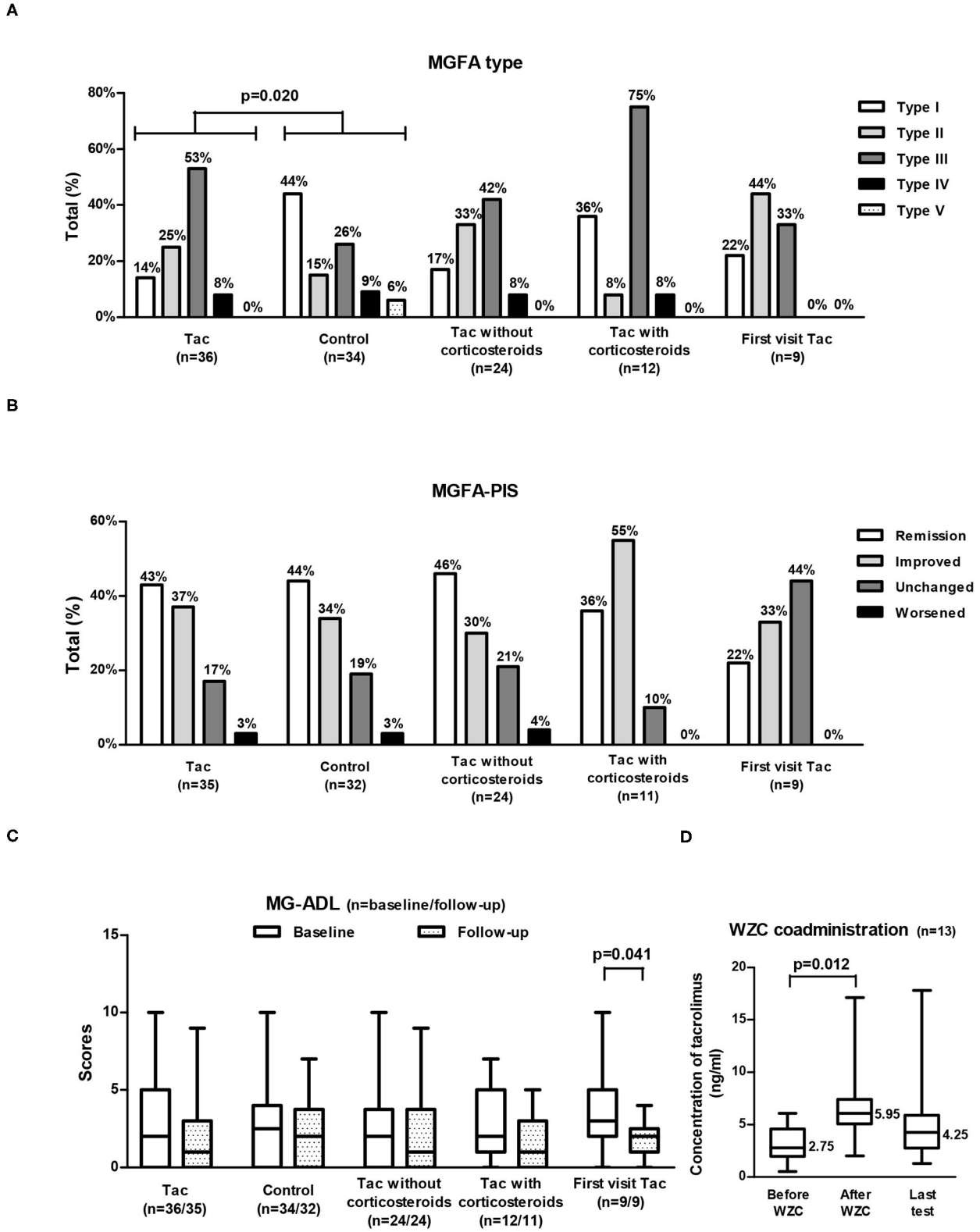


FIGURE 2 | MGFA type **(A)**, MGFA-PIS at follow-up **(B)**, and MG-ADL **(C)** in different groups of VLOMG patients and changes in tacrolimus concentrations with WZC coadministration **(D)**. MGFA type and baseline MG-ADL shared the same sample size of each group [as displayed in **(A,C)**]; MGFA-PIS and MG-ADL at follow-up were not available for the three deceased patients and they also shared the sample size presented in **(B,C)**. A total of 13 patients coadministrated WZC **(D)**. VLOMG,

(Continued)

FIGURE 2 | very-late-onset myasthenia gravis; Tac, the tacrolimus (Tac) group; control, the control group; First visit Tac, 9 newly diagnosed patients in the Tac group; MGFA, Myasthenia Gravis Foundation of America; MGFA-PIS, MGFA Post-intervention Status; MG-ADL, MG Activities of Daily Living; WZC, Wuzhi capsules. The chi-square tests were performed to compare the MGFA type or the MGFA-PIS. The Mann–Whitney U tests were utilized to compare the MG-ADL scores of different groups. For comparisons of the MG-ADL scores at baseline and follow-up or tacrolimus concentrations before and after coadministrating WZC, paired *t*-tests were conducted. Box-and-whisker plots in **(C,D)** show the medians, interquartile ranges, and min to max ranges. Significant *p* values are presented.

continuous variables were conducted between the corticosteroids and non-corticosteroids groups. And, we applied similar comparisons between ocular and generalized MG patients in the Tac group to assess the efficacy of tacrolimus in different MG types. To explore potential factors relevant to outcomes, univariable comparisons of covariates were performed between the effective and ineffective groups. Paired *t*-test was utilized to compare twice-repeated measurements.

RESULTS

Patient Characteristics

A total of 70 patients with VLOMG (30 females, 40 males) were eligible for this study, comprising 36 patients in the Tac group and 34 in the control group. Demographical and clinical characteristics are summarized in **Table 1**. The median (interquartile range) age was 71 (68–77) years at disease onset and 77 (72–81) years at the last evaluation. The median duration of follow-up was 27 (27–29) months. At the time of enrollment, the median MG–ADL score was 2 (0–4); 19 patients (27%) were newly diagnosed with MG, and 15 (21%) patients were hospitalized for MG. Distributions of MGFA classification in different groups are presented in **Figure 2A**. A total of 16 patients (23%) had thymoma or thymic hyperplasia. Antibodies were tested in 62 patients (89%), and 48 (79%) were anti-AChR antibody positive. The main treatments except for tacrolimus and WZC (these two are summarized in **Table 2**) are listed in **Table 1**.

Three subjects died during follow-up: a female (Tac group, 84 years) died from cerebral infarction together with abundant comorbidities (lung infections, coronary artery disease, Hashimoto"s thyroiditis, peripheral neuropathy, gastroesophageal reflux, atherosclerosis, and hyperuricemia). Another female patient (control group, 71 years) died from an attack of myasthenic crisis at home, and a male patient (control group, 76 years) died from tracheotomy-related death with myasthenic crisis, unresponsive wakefulness syndrome, and aspiration pneumonia. Of the 67 patients completing the final evaluation, 62 (93%) had at least one comorbidity, and 54 (77%) were taking concomitant drugs other than treating MG. The median number of comorbidities was 3 (2–4), and the three most common comorbidities were hypertension [74% (50/67)], diabetes [34% (23/67)], and dyslipidemia [24% (16/67)]. Others with incidences higher than 10% included digestive system diseases [18% (12/67)], coronary heart diseases [16% (11/67)], osteoporosis or fracture [16% (11/67)], eye diseases [16% (11/67)], other neurological diseases except for MG and cerebrovascular diseases [15% (10/67)], benign prostatic hyperplasia [13% (5/39)], liver diseases [12% (8/67)] and gynecological diseases [11% (3/28)].

Clinical Response to Tacrolimus Treatment in VLOMG

A total of 36 VLOMG patients had been treated with tacrolimus for more than 3 months, 9 of whom were newly diagnosed at enrollment. Clinical profiles and outcome measurements of the Tac group are summarized in **Table 2**, while outcomes of the first visit patients treated with tacrolimus (first visit Tac) are presented in **Table 3**. Of the 36 subjects, the median duration of MG at tacrolimus initiation was 8 (2–22) months. Age at initiation was 74 (69–79) years. A total of 23 (65%) had started before enrollment for 22 (15–32) months (minimum 5 months). By the last follow-up or discontinuation, tacrolimus treatment had lasted for 36 (27–52) months. The dosage at the final evaluation was 1.0 (1.0–1.75) mg/day, and the last blood concentration test was 4.25 (2.85–5.7) ng/ml. Nine (25%) discontinued tacrolimus since their symptoms only needed to be controlled with pyridostigmine according to the physicians' orders.

Concerning MGFA-PIS (**Figure 2B**), 43% of the Tac group achieved remission (first visit Tac: 22%), and 37% had improved (first visit Tac: 33%). The MG-ADL score of the entire Tac group remained stable during follow–up. However, among the 9 newly diagnosed patients, the MG–ADL score decreased significantly from 3 (2–5) to 2 (1–2) ($p = 0.041$, paired *t*-test) (**Figure 2C**). A total of 15 patients (43%) recalled relapse(s) or aggravation(s) [first visit Tac: 3 (33%)] after initiation of tacrolimus. Out of the 4 newly diagnosed patients with "unchanged" status, two were MGFA type I, one was MGFA type II, and the other was MGFA type III with five comorbidities (hypertension, cranial trauma, rectal polyps, hypothyroidism, and atherosclerosis). We did not discover evident differences in comparing the outcomes between generalized MG ($n = 30$) and ocular MG patients ($n = 5$). In the exploration of factors relevant to outcomes (**Table 4**), no significant differences were found in the clinical characteristics and medication details between the effective ($n = 28$) and ineffective groups ($n = 7$).

For the 13 patients (36%) who could not achieve adequate tacrolimus concentration by adjustment of dosage, WZC was prescribed. Among them, two tablets of WZC were administered together with tacrolimus every time, and the final dosage of tacrolimus was 1.5 (1–2) mg/day. The median tacrolimus concentration rose significantly ($p = 0.012$, paired *t*-test) from 2.75 (1.4–3.8) ng/ml (detected within 1 month before coadministration of WZC) to 5.95 (5.1–7.0) ng/ml (detected between the first and second months after coadministration of WZC), with an increase of 3.0 (2.4–4.5) ng/ml (**Figure 2D**). There were no patient-reported ADRs related to WZC.

The incidence of ADRs caused by tacrolimus was 23% (8/35) in the Tac group and 25% (9/36) in all the participants.

Patient-reported ADRs included elevated blood sugar, higher blood pressure, itchy skin, lung infection, headache, palpitations, involuntary hand tremor, fluctuation of prostate-specific antigen, and gaining weight, all of which were not serious and eased by dosage adjustment and/or symptomatic treatment. One patient in the control group developed severe respiratory distress after taking tacrolimus for 1 week during hospitalization. Alleviation took place after withdrawal, and tacrolimus was not used again.

Tacrolimus Without Corticosteroids vs. Tacrolimus With Corticosteroids in Treating VLOMG

Of the Tac group, 24 patients received tacrolimus without corticosteroids, and 12 coadministered corticosteroids (**Table 2**). The MGFA-PIS did not differ significantly between the two subgroups (**Figure 2B**). Remission was recorded in 46% of the non-corticosteroids group and in 36% of the corticosteroids group. The distributions of other statuses were as follows: improved [30% (non-corticosteroids) vs. 55% (corticosteroids)], unchanged [21% (non-corticosteroids) vs. 10% (corticosteroids)], and worsened [4% (non-corticosteroids) vs. 0% (corticosteroids)]. More relapse(s) or aggravation(s) during treatment occurred in patients receiving tacrolimus with corticosteroids. Notably, MGFA type III dominated the corticosteroids group (75%), while similar proportions of type II (33%) and type III (42%) were observed in the non-corticosteroids group (**Figure 2A**). There were no significant differences in other covariates, other measurements, ADRs, or tacrolimus discontinuation.

Tacrolimus vs. Other Therapies in Treating VLOMG

Comparisons of covariates between the Tac group and the control group are summarized in **Table 1**. Concerning the 70 patients, the percentages of MGFA type differed significantly ($p = 0.020$). Type III dominated the Tac group (19/36, 53%), while type I appeared the most in the control group (15/34, 44%) and 53% (8/15) of these ocular MG patients required only single-agent therapy with cholinesterase inhibitors (**Figure 2A**). The Tac group used fewer corticosteroids [using: 11 (Tac) vs. 41% (control), discontinuation: 22 (Tac) vs. 12% (control), never use: 67 (Tac) vs. 47% (control), $p = 0.015$] and fewer other immunosuppressors [using: 6 (Tac) vs. 26% (control), discontinuation: 3 (Tac) vs. 6% (control), never use: 92 (Tac) vs. 68% (control), $p = 0.038$]. Fewer patients [6% (Tac) vs. 31% (control), $p = 0.006$] in the Tac group had digestive system diseases (including peptic ulcer, intestinal microflora disorders, gastric stromal tumor, severe indigestion, stomach polyps, and rectal polyps). No differences appeared in other clinical features or sociodemographic characteristics. Neither did covariates of the first visit subgroup.

Outcome measurements of the whole cohort and subgroups are listed in **Table 3** without any significant differences. MG crisis developed in 4 patients (11%) of the Tac group and 3 (9%) of the control during follow-up. At the final evaluation, the MG-QOL-15R and SSQ scores of all the patients were 1 (0–6) and 8 (6–9), respectively; the EQ-5D-5L health value was 0.88 (0.77–1.00). The **Supplementary Table 1** displays the results of multivariate analyses. No significant associations were found between tacrolimus administration and outcomes. In the whole cohort and the MGFA > I subgroup, corticosteroids administration was associated with more relapse(s) or aggravation(s) [whole: RR = 1.382 (95% CI, 1.052 to 1.815), $p = 0.020$. MGFA > I: RR = 1.566 (95% CI, 1.182–2.076), $p = 0.002$]. A higher MGFA type was highly correlated with a lower EQ-5D-5L health value in the whole cohort [$\beta = -0.064$ (95% CI, −0.124 to −0.004), $p = 0.038$]. Patients treated with plasmapheresis were more likely to obtain lower SSQ scores [$\beta = -7.160$ (95% CI, −11.209 to −3.112), $p = 0.001$] than those who never received plasmapheresis in the MGFA > I subgroup. A history of digestive system diseases was associated with lower SSQ scores and worse MGFA-PIS in all the patients [SSQ score: $\beta = -2.328$ (95% CI, −4.450 to −0.206), $p = 0.032$; MGFA–PIS: RR = 1.292 (95% CI, 1.073–1.556), $p = 0.007$]. Analyses of patients with MGFA > I revealed similar results [SSQ score: $\beta = -2.917$ (95% CI, −5.161 to −0.673), $p = 0.012$. MGFA-PIS: RR = 1.320 (95% CI, 1.089–1.600), $p = 0.005$]. In addition, those with digestive diseases in this subgroup reported more relapse(s) or aggravation(s) than those without such diseases [RR = 1.785 (95% CI, 1.015 to 3.139), $p = 0.044$].

According to patients' description at follow-up, 45 (67%) had no ADRs, and a total of 28 ADRs were reported, consisting of 9 [25% (9/36)] caused by tacrolimus, 7 [26% (7/27)] by corticosteroids, 6 [11% (6/52)] by cholinesterase inhibitors, 3 by other drugs (anticoagulant, antiplatelet, and statins), and 3 by unclear drugs (1 skin lesion, 1 muscle pain, and 1 gastrointestinal dysfunction). The incidence of ADRs related to different drugs showed no differences between the Tac group and the control group. ADRs caused by corticosteroids included Cushing syndrome, palpitations, cataracts, elevated blood sugar, femoral head diseases, impaired kidney function, and skin lesions. Cholinesterase inhibitors caused depression, excessive saliva production, palpitations, unexplained fever, gastrointestinal dysfunction, tremor, and paresthesia.

DISCUSSION

Tacrolimus was effective for VLOMG in this study. Eighty percent of the Tac group achieved remission (43%) or improved (37%) according to the MGFA-PIS. Outcome measurements indicated tacrolimus non-inferior to other medications, consistent with one study on MG of Osserman grades III and IV (16). Although the positive effects here seemed weaker than several earlier studies that reported remission rates ≥ 64% (11, 12, 15, 16, 21), the differences could be explained by the already achieved improvement before baseline and relatively stable MG status at enrollment. Almost all the previous studies started with tacrolimus initiation, while the majority of our patients began with a notable duration of treatment; conceivably, there was less space for a positive effect to be displayed. One randomized controlled trial failing to reveal positive results faced a similar situation where the average

TABLE 2 | Clinical profiles and outcome measurements of VLOMG patients in the Tac group.

Variables	Non-corticosteroids group ($n = 24$)	Corticosteroids group ($n = 12$)	Total ($n = 36$)	*P* value
Sex				0.302
Male	13 (54%)	4 (33%)	17 (47%)	
Female	11 (46%)	8 (67%)	19 (53%)	
Clinical profiles				
Anti-AChR antibody positive	17/21 (81%)	9/10 (90%)	26/31 (84%)	1.000
Thymoma or thymic hyperplasia	6 (25%)	4 (33%)	10 (28%)	0.700
MGFA type				0.224
I	4 (17%)	1 (8%)	5 (14%)	
IIa/IIb	8 (33%)	1 (8%)	9 (25%)	
IIIa/IIIb	10 (42%)	9 (75%)	19 (53%)	
IVa/IVb	2 (8%)	1 (8%)	3 (8%)	
V	0 (0%)	0 (0%)	0 (0%)	
MG-ADL at enrollment, median (IQR)	2 (0–3.5)	2 (1–5)	2 (0–5)	0.745
Details of treatments				
Age of starting tacrolimus, median (IQR), y	76 (69–80)	74 (70–77)	74 (69–79)	0.638
Duration of MG at tacrolimus initiation, median (IQR), m	4.5 (1–17.5)	16.5 (8–23.5)	8 (2–22)	0.060
Duration from starting tacrolimus to the last follow-up or withdrawal, median (IQR), m	37 (27.5–55)	36 (21–50)	36 (27–52)	0.467
Dose of tacrolimus at the final evaluation, median (IQR), mg/day	1.0 (1.0–1.75)	1.25 (1.0–1.75)	1.0 (1.0–1.75)	0.817
The last tacrolimus concentration, median (IQR), ng/ml	4.4 (2.7–6.7)	3.9 (3.1–5.0)	4.25 (2.85–5.7)	0.630
Wuzhi capsules	9 (38%)	4 (33%)	13 (36%)	1.000
Tacrolimus concentration before taking Wuzhi capsules, median (IQR), ng/ml	2.7 (1.1–3.8)	2.8 (1.4–6.1)	2.75 (1.4–3.8)	0.569
Tacrolimus concentration after taking Wuzhi capsules, median (IQR), ng/ml	6.4 (5.1–7.0)	4.45 (2.55–6.6)	5.95 (5.1–7.0)	0.285
Change in tacrolimus concentration, median (IQR), ng/ml	4.1 (2.9–5.6)	1.3 (0.6–3.0)	3.0 (2.4–4.5)	0.071
Tacrolimus discontinuation due to the already undercontrolled symptoms[a]	5 (21%)	4 (33%)	9 (25%)	0.443
Patient-reported ADRs caused by tacrolimus	5/24 (21%)	3/11 (27%)	8/35 (23%)	1.000
Other immunosuppressors[b]	1 (4%)	2 (17%)	3 (8%)	0.253
Cholinesterase inhibitors				0.736
Never use	6 (25%)	2 (17%)	8 (22%)	
Using	11 (46%)	5 (42%)	16 (44%)	
Discontinuation	7 (29%)	5 (42%)	12 (33%)	
IVIg	4 (17%)	6 (50%)	10 (28%)	0.053
Other Chinese medicines	6 (25%)	3 (25%)	9 (25%)	1.000
Concomitant drug(s)	17 (71%)	10 (83%)	27 (75%)	0.685
Comorbidities[c]	**($n = 24$)**	**($n = 11$)**	**($n = 35$)**	
Without comorbidity	1 (4%)	1 (9%)	2 (6%)	0.536
Number of comorbidities, median (IQR)	2 (1.5–4)	2 (1–4)	2 (1–4)	0.715
Outcome measurements[c]	**($n = 24$)**	**($n = 11$)**	**($n = 35$)**	
MGFA-PIS				0.578
Remission	11 (46%)	4 (36%)	15 (43%)	
Improved	7 (30%)	6 (55%)	13 (37%)	
Unchanged	5 (21%)	1 (10%)	6 (17%)	
Worsened	1 (4%)	0 (0%)	1 (3%)	
MG-ADL, median (IQR)	1 (0–3.5)	1 (0–3)	1 (0–3)	0.756
Change of MG-ADL[d], median (IQR)	0 ([−1]–2.5)	1 (0–2)	0 ([−1]–2)	0.614

(Continued)

TABLE 2 | Continued

Variables	Non-corticosteroids group ($n = 24$)	Corticosteroids group ($n = 12$)	Total ($n = 36$)	*P* value
Have relapsed or aggravated[e]	7 (29%)	8 (73%)	15 (43%)	0.027*
MG-QOL-15R, median (IQR)	1 (0–7)	2 (0–7)	1 (0–7)	0.900
SSQ, median (IQR)	8 (6.5–9)	8 (7–9)	8 (7–9)	0.454
EQ-5D-5L health value, median (IQR)	0.91 (0.71–1.00)	0.90 (0.77–1.00)	0.90 (0.74–1.00)	0.730

[a]*Symptoms can be controlled with only cholinesterase inhibitors according to the physicians' orders.*
[b]*Included 1 mycophenolate mofetil, 1 rituximab, and 1 cyclophosphamide.*
[c]*A female patient in the Tac group died during follow-up. Information on her comorbidities, ADRs, and outcome measurements were not available. Of the 35 patients completing the last follow-up, 17 (49%) answered in person, 13 (54%) answered in the corticosteroids group, and 4 (36%) answered in the non-corticosteroids group (p = 0.328, no significant differences).*
[d]*Subtract MG-ADL score at enrollment from the score at the last follow-up.*
[e]*This item was evaluated by the patients' description after being asked "Have you ever relapsed or aggravated since (the date at enrollment)".*
AChR, acetylcholine receptor; MG, Myasthenia Gravis; MGFA, Myasthenia Gravis Foundation of America; IQR, interquartile range; IVIg, intravenous immunoglobulin; MG-ADL, MG Activities of Daily Living; IQR, interquartile range; EQ-5D-5L, five-level EuroQol five-dimensional questionnaire; MG-QOL-15R, revised 15-item Myasthenia Gravis Quality of Life scale; SSQ, simple single question; MGFA-PIS, MGFA Post-intervention Status.
**Statistically significant.*

TABLE 3 | Outcome measurements of VLOMG patients in the whole cohort and subgroups[a].

Outcome	Whole ($n = 67$)				First visit ($n = 18$)			
	Control group ($n = 32$)	Tac group ($n = 35$)	Total ($n = 67$)	*P* value	Control group ($n = 9$)	Tac group ($n = 9$)	Total ($n = 18$)	*P* value
MGFA-PIS				0.995				0.214
Remission	14 (44%)	15 (43%)	29 (43%)		5 (56%)	2 (22%)	7 (39%)	
Improved	11 (34%)	13 (37%)	24 (36%)		3 (33%)	3 (33%)	6 (33%)	
Unchanged	6 (19%)	6 (17%)	12 (18%)		1 (11%)	4 (44%)	5 (28%)	
Worsened	1 (3%)	1 (3%)	2 (3%)		0 (0%)	0 (0%)	0 (0%)	
MG-ADL, median (IQR)	2 (0–3.5)	1 (0–3)	1 (0–3)	0.690	1 (0–2)	2 (1–2)	1.5 (0–2)	0.272
Change of MG-ADL[b], median (IQR)	−1 ([−2]−1)	0 ([−2]−1)	−1 ([−2]−1)	0.944	1 (1–2)	1 (0–3)	1 (0–3)	0.755
Have relapsed or aggravated[c]	17 (53%)	15 (43%)	32 (48%)	0.401	3 (33%)	3 (33%)	6 (33%)	1.000
MG crisis attack during follow-up	3/34 (9%)	4/36 (11%)	7/70 (10%)	0.750	1/10 (10%)	0/9 (0%)	1/19 (5%)	0.330
MG-QOL-15R, median (IQR)	2 (0–6)	1 (0–7)	1 (0–6)	0.737	3 (0–7)	1 (1–2)	1 (0–6)	0.752
SSQ, median (IQR)	7.5 (6–9)	8 (7–9)	8 (6–9)	0.400	8 (7–10)	9 (8–9)	8.5 (7–9)	0.964
EQ-5D-5L health value, median (IQR)	0.88 (0.80–0.96)	0.90 (0.74–1.00)	0.88 (0.77–1.00)	0.790	0.83 (0.83–0.91)	1.00 (0.88–1.00)	0.89 (0.83–1.00)	0.079

[a]*Outcome measurements of the three deceased patients were not available. Of the 67 patients completing the last follow-up, 36 (54%) answered in person, 19 (60%) in the control group, and 17 (49%) in the Tac group (p = 0.376). There were also no significant differences in respondents in the two subgroups.*
[b]*Subtract MG-ADL score at enrollment from the score at the last follow-up.*
[c]*This item was evaluated by the patients' description after being asked "Have you ever relapsed or aggravated since (the date at enrollment)." Whole, 67 patients completed the last follow-up except 3 deceased; First visit, 19 patients who first visited our institution and were newly diagnosed with MG at enrollment; MGFA > 1, 50 patients whose MGFA type was higher than I; MG-ADL, MG Activities of Daily Living; IQR, interquartile range; EQ-5D-5L, five-level EuroQol five-dimensional questionnaire; MG-QOL-15R, revised 15-item Myasthenia Gravis Quality of Life scale; SSQ, simple single question; MGFA-PIS, MGFA Post-intervention Status.*

MG-ADL at entry was only 1.8 (29). We analyzed the first visit Tac subgroup to remove the intervention of treatments before baseline, where MG-ADL decreased significantly during follow-up. Advantages of tacrolimus in generalized MG could be further suggested regarding the higher MGFA type in the Tac group and the marked proportion of ocular MG patients treated with cholinesterase inhibitors alone in the control group. It also agreed with the clinical consensus that tacrolimus works well as an add-on therapy for those who were intolerant or did not respond well enough to corticosteroids. Moreover, high quality of life in VLOMG patients treated with tacrolimus was observed in this study, with MG-QOL-15R close to the remission patients and EQ-5D-5L health utility value matching MGFA type I (30, 31). Covariates and outcomes did not display statistical differences between tacrolimus with and without corticosteroids in this study. Nonetheless, the results should be interpreted carefully and conservatively according to the small sample size, since the effects of some non-statistically significant differences (such as MGFA type) could exist. Though it was insufficient to conclude that the two therapies held comparable efficacy, at least part of the VLOMG population in China could benefit from tacrolimus monotherapy, achieving relatively

TABLE 4 | Characteristics of VLOMG patients in the Tac group according to clinical outcome.

Variables	Ineffective group ($n = 7$)	Effective group ($n = 7$)	Total ($n = 35$)	*P* value
Sex				0.612
Male	4 (57%)	13 (46%)	17 (49%)	
Female	3 (43%)	15 (54%)	18 (51%)	
Clinical profiles				
Anti-AChR antibody positive	7/7 (100%)	19/23 (83%)	26/30 (87%)	0.236
Thymoma or thymic hyperplasia	1 (14%)	9 (32%)	10 (29%)	0.350
MGFA type				0.841
I	1 (14%)	4 (14%)	5 (14%)	
IIa/IIb	1 (14%)	8 (29%)	9 (26%)	
IIIa/IIIb	4 (57%)	14 (50%)	18 (51%)	
IVa/IVb	1 (14%)	2 (7%)	3 (9%)	
MG-ADL at enrollment, median (IQR)	2 (2–5)	2 (0–4.5)	2 (0–5)	0.501
Details of tacrolimus medication				
Age of starting tacrolimus, median (IQR), y	70 (69–77)	74 (70–80)	74 (69–79)	0.405
Duration of MG at tacrolimus initiation, median (IQR), m	2 (1–11)	11 (2.5–23)	9 (2–22)	0.200
Duration from starting tacrolimus to the last follow-up or withdrawal, median (IQR), m	28 (25–44)	40.5 (27–54)	36 (27–52)	0.454
Dose of tacrolimus at the final evaluation, median (IQR), mg/day	1.75 (1.0–2.5)	1.0 (1.0–1.5)	1.0 (1.0–2.0)	0.233
The last tacrolimus concentration, median (IQR), ng/ml	4.65 (3.1–7.2)	4.25 (2.7–5.0)	4.25 (2.85–5.7)	0.579
Comorbidities				
Without comorbidity	1 (14%)	1 (4%)	2 (6%)	0.275
Number of comorbidities, median (IQR)	2 (1.5–4)	2 (1–4)	2 (1–4)	0.220

AChR, acetylcholine receptor; MG, Myasthenia Gravis; MGFA, Myasthenia Gravis Foundation of America; IQR, interquartile range; MG-ADL, MG Activities of Daily Living; IQR, interquartile range; EQ-5D-5L, five-level EuroQol five-dimensional questionnaire; MG-QOL-15R, revised 15-item Myasthenia Gravis Quality of Life scale; SSQ, simple single question.

satisfying improvement or maintenance while avoiding the ADRs of steroids. Similar comparisons were rare except for one retrospective study of generalized MG showing coadministration with corticosteroids better (32). And it was not clear whether to use tacrolimus directly as a single drug, or to take corticosteroids first to stabilize and then tacrolimus alone for maintenance. Studies with larger samples and longer follow-up are required to evaluate the effectiveness of tacrolimus monotherapy. The only measurement showing differences was more relapse(s) or aggravation(s) in the corticosteroids group, which is reasonable since steroids are usually the first-line treatment for more severe MG (2). It was worth noting that the recurrence rate of the whole Tac group was relatively high. However, this could be explained from the following four aspects. First, the incidence of relapse(s) or aggravation(s) in the control group was similarly high, indicating reasons other than tacrolimus to account for this abnormity. Second, older age of onset was discovered to be a remarkable predictor for OMG generalization (33). Third, the definition of relapse(s) or aggravation(s) was not consistent across studies. Lower rates were reported in studies where relapse was defined as the reappearance of extraocular symptoms (33) and aggravation as increases of quantitative scores (13, 32), while we counted the recurrence of ocular symptoms and used more subjective assessment. Fourth, our patients' compliance was probably lower owing to COVID-19 pandemic where visiting doctors and getting prescription drugs were restricted. We found some patients discontinued their medications without physicians' orders. For the future researches and clinical practice, we suggest including ocular disorders in the criteria of relapse due to the deterioration of life quality; VLOMG patients should be followed up more closely and online mode is recommended. Although we identified no obvious difference in univariable comparisons of outcomes between patients with generalized and ocular MG, the two subgroups were unbalanced (30 patients vs. 5 patients) so the statistical power might be unconvincing to draw conclusions. Clinical characteristics and medication details did not appear to affect the effectiveness of tacrolimus, possibly because of the small sample size. Further investigations are needed to disclose the features of VLOMG patients who are more suitable to receive tacrolimus treatment. In short, considering the favorable results observed in our study and the devastating ADRs of corticosteroids in the elderly (23), tacrolimus without corticosteroids could be one of the treatment options for VLOMG.

For the VLOMG patients with unsatisfactory tacrolimus concentrations, coadministering WZC helped them reach the recommendation of 2 to 9 ng/ml with no obvious side effects (23). Other products of Wuweizi (Schisandra sphenanthera)

were not used in this study, thus excluding the effect of non-WZC drugs on plasma levels. Active components of WZC compete with tacrolimus to bind metabolic enzymes (CYP3A5 and CYP3A4) and inhibit the *P*-glycoprotein-mediated efflux of tacrolimus, thereby, reducing the intestinal first-pass effect and remarkably increasing the concentration (17). Compared with increasing the dosage of tacrolimus, prescribing WZC did not merely achieve similar efficacy but provided extra profits of preventing hepatotoxicity and reducing the financial burden (19). Neither tacrolimus nor WZC is covered by basic medical insurance in China. Assuming 30 days a month and considering the prices of drugs used by our patients, taking tacrolimus at 3 mg/day costs 2,700 RMB (423.63 USD) per month, while tacrolimus 1.5 mg/day plus WZC 4 capsules/day costs only 1,470 RMB (230.64 USD). Notably, the average pension for retirees was estimated to be 3,500.60 RMB (549.24 USD) per month in 2021 according to the Ministry of Human Resources and Social Security of China, which means that elevating tacrolimus dosage could bring a heavy burden to VLOMG patients. Instead, usage of WZC economically benefits elderly patients who metabolize tacrolimus more quickly. Although the small sample size of WZC takers in this study limited the validity, our observations complemented the previous study in younger MG patients aged 36 (27–50) years (18).

In multivariable analyses, a history of digestive diseases was identified to be associated with worse MGFA-PIS and lower SSQ scores. Although earlier studies on MG rarely reported gastrointestinal diseases, these studies did not examine patients with VLOMG. While polyps, gastric stromal tumors, and indigestion showed no direct relevance to MG, a higher incidence of peptic ulcer in the control group indicated certain hazard factors. Corticosteroids were suggested given their contribution to ulcer development. In addition, dysregulated gut microbiota seemed to affect MG manifestations by promoting an imbalance in T cell populations (34). A higher MGFA type was found to be correlated with a lower EQ-5D-5L health value, in accordance with the foregoing findings (30). Plasmapheresis seemed to be correlated with a worse prognosis, reflecting more serious disease conditions (two with MGFA III, one with MGFA IV), which led to worse outcomes. The small sample size and lack of plasmapheresis in the Tac group limited further inference.

Most ADRs caused by tacrolimus in our study were mild and easily relieved, which has also been reported by the previous studies, while the incidence varied (10–13, 29). Weight gaining, an unreported ADR, could be explained by the diabetogenicity of calcineurin inhibitors (35). This also suggests blood glucose be more closely monitored and carefully balanced in VLOMG patients after taking tacrolimus, considering the higher prevalence of diabetes or prediabetes in the elderly and the well-recognized ADR of hyperglycemia. Fluctuation of prostate-specific antigen has not been reported, but sirolimus, which has a similar structure to that of tacrolimus, was found to be associated with a decrease of prostate-specific antigen in the kidney recipients (36). As expected, almost all the participants were affected by comorbidities, and higher incidences of chronic diseases were displayed (5, 7, 32). A total of 77% were taking concomitant drugs such as beta-blockers and statins, both of which were reported to worsen MG, although these reactions were not observed in our patients (37). As earlier studies showed that more than two comorbidities were related to poorer outcomes (8), the number of comorbidities in our patients was noteworthily 3 (2–4). One in the Tac group died of cerebral infarction, confirming the destructive impact of comorbidities burden. The occurrence of MG crisis during follow-up in our study was higher than the reported Chinese data which covered all the age groups (7). For the two dying of MG crisis in the control group, there seemed no direct associations with medications regarding the normal therapies they received. Failure to quickly identify the impending respiratory paralysis and complex comorbid conditions could explain the death of an outbreak at home; the other patient died from tracheotomy-related death together with MG crisis, unresponsive wakefulness syndrome, and pneumonia, all being hazard factors for tracheotomy complications (38). We need to focus more on the prevention of myasthenia crisis, which is typically precipitated by poor control of generalized MG. In summary, VLOMG patients demand conscientious and individualized management, and more specific treatment guidelines and consensus are needed.

This study has several limitations. First, this is a single-center retrospective observational study with relatively small sample size. More controlled studies and high-quality RCTs are necessary to confirm our findings. Second, follow-up was conducted through telephone interviews, and only patient-reported outcomes were adopted, which introduced more recall bias and reduced the objectivity of our study. However, the lack of face-to-face follow-up seemed acceptable under the COVID-19 pandemic, and patient-reported outcomes provided more comprehensive depictions of the treatment impact. For a more comprehensive evaluation, combining physician examination scales with patient-reported scales is a superior option. Third, the rate of contact loss was relatively high, which could cause the measured outcomes to be worse than the actual outcomes since patients with less severe symptoms tended not to keep in touch with their doctors. Conversely, lacking measurements of the deceased may lead to a better estimation of outcomes, as patients who died during follow-up were probably under poor conditions.

In conclusion, tacrolimus is effective and safe in the treatment of VLOMG. Our results also show that tacrolimus monotherapy without corticosteroids could be one choice for VLOMG initial and maintenance treatment, especially for those who cannot tolerate or do not want to use corticosteroids and other immunosuppressors. More studies are needed to confirm the efficacy of tacrolimus monotherapy and to select patients with VLOMG who are suitable for such treatment. Coadministering Wuzhi capsules can effectively and safely improve tacrolimus concentrations when needed. Patients with higher MGFA types or comorbidities need more frequent monitoring and cautious management.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board and Ethics Committee at Peking University First Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YZhe contributed with drafting and revising the manuscript, study concept and design, and acquisition of data. XY contributed with drafting and revising the manuscript, study design, acquisition of data, and statistical analysis. CZ, RL, HJ, HH, FL, YZha, and YY contributed with the acquisition of data. ZW contributed with revising the manuscript. FG contributed with revising the manuscript, study concept and design, and interpretation of the data. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Scientific Research Seed Fund of Peking University First Hospital (2018SF033). Funding bodies did not play a role in the collection, analysis, and interpretation of data. Funding bodies did not contribute to the writing of this manuscript.

ACKNOWLEDGMENTS

The authors would thank all the patients and their families for their support of this research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.843523/full#supplementary-material

REFERENCES

1. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol.* (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3
2. Gilhus NE, Tzartos S, Evoli A, Palace J, Burns TM, Verschuuren JJGM. Myasthenia gravis. *Nat Rev Dis Primers.* (2019) 55:1–19. doi: 10.1038/s41572-019-0079-y
3. Cortés-Vicente E, Álvarez-Velasco R, Segovia S, Paradas C, Casasnovas C, Guerrero-Sola A, et al. Clinical and therapeutic features of myasthenia gravis in adults based on age at onset. *Neurology.* (2020) 94:e1171–80. doi: 10.1212/WNL.0000000000008903
4. Barnett C, Bril V. New insights into very-late-onset myasthenia gravis. *Nat Rev Neurol.* (2020). 16:299–300. doi: 10.1038/s41582-020-0345-3
5. Vijayan J, Menon D, Barnett C, Katzberg H, Lovblom LE, Bril V. Clinical profile and impact of comorbidities in patients with very-late-onset myasthenia gravis. *Muscle Nerve.* (2021) 62:462–6. doi: 10.1002/mus.27369
6. Murai H, Yamashita N, Watanabe M, Nomura Y, Motomura M, Yoshikawa H, et al. Characteristics of myasthenia gravis according to onset-age: Japanese nationwide survey. *J Neurol Sci.* (2011) 305:97–102. doi: 10.1016/j.jns.2011.03.004
7. Chen J, Tian DC, Zhang C, Li Z, Zhai Y, Xiu Y, et al. Incidence, mortality, and economic burden of myasthenia gravis in China: a nationwide population-based study. *Lancet Reg Health Western Pacific.* (2020) 5:100063. doi: 10.1016/j.lanwpc.2020.100063
8. Misra UK, Kalita J, Singh VK, Kumar S, A. study of comorbidities in myasthenia gravis. *Acta Neurol Belg.* (2020) 120:59–64. doi: 10.1007/s13760-019-01102-w
9. Alkhawajah NM, Oger J. Treatment of myasthenia gravis in the aged. *Drugs Aging.* (2015) 32:689–97. doi: 10.1007/s40266-015-0297-2
10. Zhao CB, Zhang X, Zhang H, Hu XQ, Lu JH, Lu CZ, et al. Clinical efficacy and immunological impact of tacrolimus in Chinese patients with generalized myasthenia gravis. *Int Immunopharmacol.* (2011) 11:519–24. doi: 10.1016/j.intimp.2010.12.012
11. Kim YH, Shin HY, Kim SM. Long-term safety and efficacy of tacrolimus in *Myasthenia gravis. Yonsei Med J.* (2019) 60:633–9. doi: 10.3349/ymj.2019.60.7.633
12. Fan Z, Li Z, Shen F, Zhang X, Lei L, Su S, et al. Favorable effects of tacrolimus monotherapy on myasthenia gravis patients. *Front Neurol.* (2020) 11:1–10. doi: 10.3389/fneur.2020.594152
13. Zhou L, Liu W, Li W, Li H, Zhang X, Shang H, et al. Tacrolimus in the treatment of myasthenia gravis in patients with an inadequate response to glucocorticoid therapy: randomized, double-blind, placebo-controlled study conducted in China. *Ther Adv Neurol Disord.* (2017) 10:315–25. doi: 10.1177/1756285617721092
14. Nagane Y, Utsugisawa K, Obara D, Kondoh R, Terayama Y. Efficacy of low-dose FK506 in the treatment of myasthenia gravis—a randomized pilot study. *Eur Neurol.* (2005) 53:146–50. doi: 10.1159/000085833
15. Duan W, Peng Y, Jin W, Ouyang S, Yang H. Tacrolimus as single-agent immunotherapy and minimal manifestation status in nonthymoma *Myasthenia gravis. J Immunol Res.* (2021) 2021:1–7. doi: 10.1155/2021/9138548
16. Zhao LN, Liang Y, Fang XJ, Liu XM, Jiang QL, Wang SS, et al. Efficacy and safety of tacrolimus in Osserman grade III and Osserman grade IV myasthenia gravis. *Clin Neurol Neurosurg.* (2018) 173:70–6. doi: 10.1016/j.clineuro.2018.07.023
17. Han Y, Zhou H, Cai J, Huang J, Zhang J, Shi SJ, et al. Prediction of tacrolimus dosage in the early period after heart transplantation: a population pharmacokinetic approach. *Pharmacogenomics.* (2019) 20:21–35. doi: 10.2217/pgs-2018-0116
18. Peng Y, Jiang F, Zhou R, Jin W, Li Y, Duan W, et al. Clinical evaluation of the efficacy and safety of co-administration of wuzhi capsule and tacrolimus in adult Chinese patients with myasthenia gravis. *Neuropsychiatr Dis Treat.* (2021) 17:2281–9. doi: 10.2147/NDT.S319500
19. Qin XL, Chen X, Zhong GP, Fan XM, Wang Y, Xue XP, et al. Effect of Tacrolimus on the pharmacokinetics of bioactive lignans of Wuzhi tablet (*Schisandra sphenanthera* extract) and the potential roles of CYP3A and *P*-gp. *Phytomedicine.* (2014) 21:766–72. doi: 10.1016/j.phymed.2013.12.006
20. Sussman J, Farrugia ME, Maddison *P*, Hill M, Leite MI, Hilton-Jones D. Myasthenia gravis: association of british neurologists' management guidelines. *Pract Neurol.* (2015) 15:199–206. doi: 10.1136/practneurol-2015-001126
21. Ponseti JM, Gamez J, Azem J, López-Cano M, Vilallonga R, Armengol M. Tacrolimus for myasthenia gravis: a clinical study of 212 patients. *Ann N Y Acad Sci.* (2008) 1132:254–63. doi: 10.1196/annals.1405.000

22. Kanai T, Uzawa A, Kawaguchi N, Himuro K, Oda F, Ozawa Y, et al. Adequate tacrolimus concentration for myasthenia gravis treatment. *Eur J Neurol.* (2017) 24:270–5. doi: 10.1111/ene.13189
23. Morren J, Li Y. Maintenance immunosuppression in myasthenia gravis, an update. *J Neurol Sci*. (2020) 410:116648. doi: 10.1016/j.jns.2019.116648
24. Benatar M, Sanders DB, Burns TM, Cutter GR, Guptill JT, Baggi F, et al. Recommendations for myasthenia gravis clinical trials. *Muscle Nerve.* (2012) 45:909–17. doi: 10.1002/mus.23330
25. Burns TM, Sadjadi R, Utsugisawa K, Gwathmey KG, Joshi A, Jones S, et al. International clinimetric evaluation of the MG-QOL15, resulting in slight revision and subsequent validation of the MG-QOL15r. *Muscle Nerve.* (2016) 54:1015–22. doi: 10.1002/mus.25198
26. Abraham A, Breiner A, Barnett C, Katzberg HD, Bril V. The utility of a single simple question in the evaluation of patients with myasthenia gravis. *Muscle Nerve.* (2018) 57:240–4. doi: 10.1002/mus.25720
27. Luo N, Liu G, Li M, Guan H, Jin X, Rand-Hendriksen K. Estimating an EQ-5D-5L value set for China. *Value Health.* (2017) 20:662–9. doi: 10.1016/j.jval.2016.11.016
28. Lee J, Chuen ST, Kee SC. A practical guide for multivariate analysis of dichotomous outcomes. *Ann Acad Med Singapore.* (2009) 38:714–9.
29. Yoshikawa H, Kiuchi T, Saida T, Takamori M. Randomised, double-blind, placebo-controlled study of tacrolimus in myasthenia gravis. *J Neurol Neurosurg Psychiatry*. (2011) 82:970–7. doi: 10.1136/jnnp-2011-300148
30. Barnett C, Bril V, Bayoumi AM. EQ-5D-5L and SF-6D health utility index scores in patients with myasthenia gravis. *Eur J Neurol.* (2019) 26:452–9. doi: 10.1111/ene.13836
31. Luo Y, Dong X, Peng Y, Cui B, Yan C, Jin W, et al. Evaluation of outcome measures for myasthenia gravis subgroups. *J Clin Neurosci.* (2021) 91:270–5. doi: 10.1016/j.jocn.2021.07.020
32. Zhang C, Bu B, Yang H, Wang L, Liu W, Duan RS, et al. Immunotherapy choice and maintenance for generalized myasthenia gravis in China. *CNS Neurosci Therap.* (2020) 26:1241–54. doi: 10.1111/cns.13468
33. Wang L, Zhang Y, He M. Clinical predictors for the prognosis of myasthenia gravis. *BMC Neurol.* (2017) 17:1–6. doi: 10.1186/s12883-017-0857-7
34. Rinaldi E, Consonni A, Guidesi E, Elli M, Mantegazza R, Baggi F. Gut microbiota and probiotics: novel immune system modulators in myasthenia gravis? *Ann N Y Acad Sci.* (2018) 1413:49–58. doi: 10.1111/nyas.13567
35. Wissing KM, Pipeleers L. Obesity, metabolic syndrome and diabetes mellitus after renal transplantation: prevention and treatment. *Transplant Rev.* (2014) 28:37–46. doi: 10.1016/j.trre.2013.12.004
36. Chamie K, Ghosh PM, Koppie TM, Romero V, Troppmann C, DeVere White RW. The effect of sirolimus on prostate-specific antigen (PSA) levels in male renal transplant recipients without prostate cancer. *Am J Transp.* (2008) 8:2668–73. doi: 10.1111/j.1600-6143.2008.02430.x
37. Sheikh S, Alvi U, Soliven B, Rezania K. Drugs that induce or cause deterioration of myasthenia gravis: an update. *J Clin Med*. (2021) 10:1537. doi: 10.3390/jcm10071537
38. Jotic AD, Milovanovic JP, Trivic AS, Folic MM, Krejovic-Trivic SB, Radin ZZ, et al. Predictors of complications occurrence associated with emergency surgical tracheotomy. *Otolaryngol Head Neck Surg.* (2021) 164:346–52. doi: 10.1177/0194599820947001

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 07 April 2022
doi: 10.3389/fneur.2022.858628

Association Between Clinical Factors and Result of Immune Checkpoint Inhibitor Related Myasthenia Gravis: A Single Center Experience and Systematic Review

Jiayu Shi[1], *Ying Tan*[1], *Yangyu Huang*[1], *Ke Li*[1], *Jingwen Yan*[1], *Yuzhou Guan*[1]* *and Li Zhang*[2]

[1] *Department of Neurology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China,* [2] *Department of Respiratory and Critical Care, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China*

OPEN ACCESS

Edited by:
Nils Erik Gilhus,
University of Bergen, Norway

Reviewed by:
Piotr Szczudlik,
Medical University of Warsaw, Poland
Timothy John Day,
Royal Melbourne Hospital, Australia

***Correspondence:**
Yuzhou Guan
guanyz001@163.com

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *20 January 2022*
Accepted: *15 March 2022*
Published: *07 April 2022*

Citation:
Shi J, Tan Y, Huang Y, Li K, Yan J, Guan Y and Zhang L (2022) Association Between Clinical Factors and Result of Immune Checkpoint Inhibitor Related Myasthenia Gravis: A Single Center Experience and Systematic Review. Front. Neurol. 13:858628. doi: 10.3389/fneur.2022.858628

Background: Neurological immune-related adverse events (nirAEs) are rare toxicities of immune-checkpoint inhibitors (ICI). With the increase use of ICIs, incidence of nirAEs is growing, among which ICI related MG (irMG) is causing high fatality rate. Given the limited evidence, data from a large cohort of patients with irMG is needed to aid in recognition and management of this fatal complication.

Objective: This study aimed to summarize clinical characteristics of irMG and explore predictors of irMG clinical outcome.

Methods: We summarized our institution's patients who were diagnosed as irMG between Sep 2019 and Oct 2021. We systematically reviewed the literature through Oct 2021 to identify all similar reported patients who met inclusion criteria. As the control group, patients with idiopathic MG were used. We collected data on clinical features, management, and outcomes of both irMG and idioMG cases. Further statistical analysis was conducted.

Results: Sixty three irMG patients and 380 idioMG patients were included in the final analysis. For irMG patients, six were from our institution while the rest 57 were from reported cases. The average age of irMG patients is 70.16 years old. Forty three were male. Average time from first ICI injection to symptom onset was 5.500 weeks. Eleven patients had a past history of MG. Higher MGFA classification and higher QMGS rates were observed in irMG patients compared to idioMG patients. For complication, more irMG patients had myositis or myocarditis overlapping compared to idioMG patients. The most commonly used treatment was corticosteroids for both idioMG and irMG. Twenty one patients (35%) with irMG had unfavorable disease outcome. Single variate and multivariate binary logistic regression proved that association with myocarditis, high MGFA classification or QMGS rates at first visit were negatively related to disease outcome in irMG patients.

Conclusion: irMG is a life-threatening adverse event. irMG has unique clinical manifestations and clinical outcome compared to idioMG. When suspicious, early evaluation of MGFA classification, QMGS rates and myositis/myocarditis evaluation are recommended.

Keywords: myasthenia gravis, MGFA, QMG, immune-related adverse effects, immune checkpoint inhibitors

INTRODUCTION

Immune checkpoint inhibitors (ICIs) are regarded as effective treatments for different types of advanced cancers (1). Despite impressive benefits observed from using ICIs, these treatments may be associated with serious immune-related adverse events (irAEs) caused by the induction of off-target inflammatory and autoimmune responses (1, 2).

ICI-related neurological adverse events are relatively infrequent; however, pooled analyses have shown that they are associated with increased morbidity and mortality (3–5). Myasthenia gravis (MG) is an autoimmune disorder mediated by autoantibodies, including anti-acetylcholine receptor (AChR) or anti-muscle associated receptor tyrosine kinase (MUSK) antibodies that target the neuromuscular junction (6). MG induced by ICI treatment or ICI-induced relapse of pre-existing MG is known as immune-related MG (irMG) (7). The incidence rate of irMG is 0.1%−0.2% according to the current literature (2, 8). Because of the low incidence rate and limited number of described cases, characterization of clinical features and prediction of disease outcome for irMG is difficult based on a patient's clinical manifestations. In this study, we described the clinical features of 63 patients with irMG and aimed to identify possible factors that may be useful for predicting irMG prognosis.

MATERIALS AND METHODS

Patients

Patients who were diagnosed with irMG at PUMCH between September 2019 and October 2021 were included in the study cohort. We also searched the PubMed and EMBASE databases through October 2021 for case reports, case series, and observational studies that described patients with cancer and MG who received ICI treatment. The database searches did not include language or study design restrictions. Titles and abstracts were screened by two independent investigators to identify potentially relevant articles. Then, the full text of each selected article was retrieved and reviewed. A detailed clinical description of each patient was generated. The keywords included in the search were ("immune checkpoint inhibitors" OR "nivolumab" OR "ipilimumab" OR "pebrolizumab" OR "avelumab" OR "durvalumab" OR "atezolizumab" OR 'anti-PD-1" OR "anti-PD-L1" OR "anti-CTLA-4") AND ("immune related MG" OR "irMG" OR "MG" OR "Myasthenia Gravis" OR "ocular Myasthenia Gravis"). An inclusion diagram of patients is shown in **Figure 1**. The quality appraisal of the reported cases from the literature is shown in **Supplementary Table 3**. The inclusion criteria for both the PUMCH patients and cases identified in the literature included: (1) diagnosed with cancer; (2) any type of ICI used before MG onset or relapse; (3) definite or probable diagnosis of new-onset MG or deterioration of symptoms of well-controlled MG; and (4) a detailed description of the patient's clinical course was available. A definite diagnosis of MG was based on the presentation of ocular and/or systemic muscle weakness and at least one of the following criteria: (1) elevated titers of anti-AChR or anti-MUSK antibodies, (2) findings suggestive of MG in electrodiagnostic tests, (3) positive edrophonium test, or (4) positive ice pack test. A probable diagnosis of MG was made based on high clinical suspicion by the neurologist's report that confirmed the diagnosis of MG. We excluded patients with thymoma as an indicator for ICI treatment.

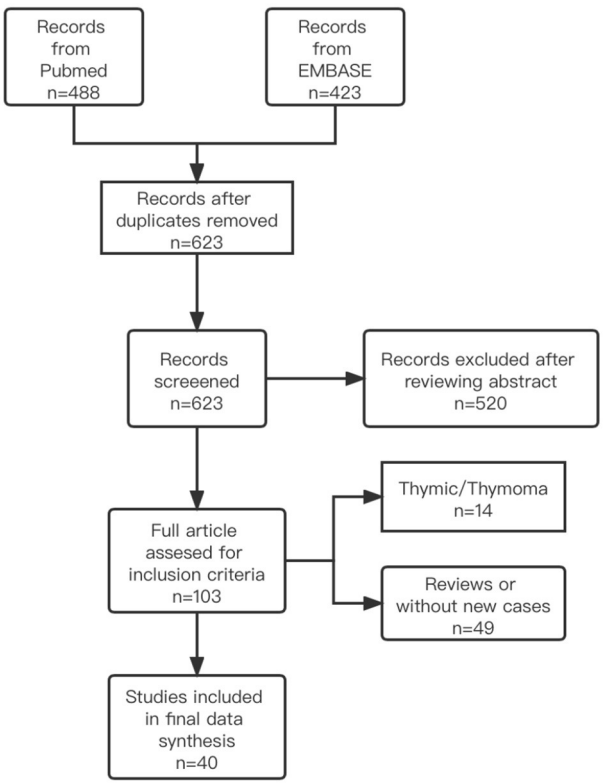


FIGURE 1 | Flow gram describing the systematic search and study selection process.

Controls

We also studied patients with idiopathic MG (idioMG), who were diagnosed at the PUMCH Neurology Department and registered

at PUMCH MG registry as the control group. We have excluded idioMG patients with thymoma.

Methods

For both the PUMCH and literature identified patients, we extracted variables for patient demographics and baseline characteristics, including age, sex, type of ICI, indication of ICI, cancer staging, time between disease onset and first and last ICI injection, and severity of irAEs. We assessed the clinical severity of irMG using the Myasthenia Gravis Foundation of America (MGFA) classification system. For PUMCH patients, the quantitative myasthenia gravis score (QMGS) was determined by a trained neurologist at each patient's first visit. The QMGS was also collected for literature-identified patients if available. Data on clinical manifestations of MG (ptosis, diplopia, dyspnea, limb weakness and dysphagia); titer results for anti-AchR, anti-MUSK, and anti-titin antibodies; and overlap with myositis, myocarditis, or other system irAEs were also collected if available. Myositis was defined as elevated creatine kinase (CK) levels after disease onset. Myocarditis was defined as elevated cardiac troponin I levels, dynamic changes in electrocardiogram data, or symptoms of acute coronary artery syndrome. An unfavorable outcome was defined as tracheotomy, endotracheal intubation, or death directly caused by ICI-related MG.

Statistical Analysis

Baseline characteristics of irMG group were evaluated using frequencies and percentages for categorical data, while median and range were used to describe continuous data. Comparisons of categorical variables between control group and patient group were tested for significance using the x2 test. Continuous variables were compared using the Mann-Whitney U test. We performed single variate binary logistic regression analyses to determine the odds ratios (ORs) for associations between certain clinical or demographic factors and risk of unfavorable outcomes for irMG. Factors that were significantly associated with an unfavorable outcome were analyzed together in a multivariate binary logistic regression model. This analysis was performed with the maximal level of adjustment. All tests were 2-sided, and Bonferroni correction was applied to the α level to adjust for multiple comparisons. Bonferroni-adjusted *p* values are reported in the tables. Statistical analyses were carried out using the SPSS 24.0 statistical package (SPSS; Chicago, IL, USA). The study was approved by the local ethics committee.

RESULTS

For irMG group, six patients from PUMCH were diagnosed with irMG. Of 623 unique articles from the literature, 40 publications describing 57 patients met the inclusion criteria (3, 6, 9–48). Therefore, a total of 63 patients were included in our final analysis. For idioMG group, we included 380 patients from PUMCH MG registry during the same period.

irMG Patient Demographic and Baseline Characteristics

irMG patients' characteristics are shown in detail in **Supplementary Table 1**. A summary of demographic and baseline characteristics is shown in **Table 1**. The most common indication for ICIs was melanoma followed by urethral and lung carcinoma. The majority of patients had progressive tumor staging, and inhibitors of programmed cell death 1 (PD-1) were the most commonly applied therapeutics in the total cohort. The median time from ICI injection to symptom onset was 5 weeks, while the median time from the last ICI injection to symptom onset was 10 days. The severity of irAEs in the majority of patients in our cohort were classified at level IV. More than 60% of the patients had irAEs involving other systems. The most commonly involved system was cardiovascular system, followed by digestive system. Skin and hematological system irAEs were also observed.

irMG Characteristics and Comparison With idioMG Group

The patients' irMG characteristics, treatments, and outcomes are shown in detail in **Supplementary Table 2**. A comparison of irMG and idioMG characteristics are shown in **Table 2**. Among the 63 patients identified with irMG, 11 had a past history of well-managed MG and presented with a flare-up of MG after ICI initiation. The MGFA classification and QMGS rates clearly demonstrated that the disease was more severe in patients with irMG than in patients with idioMG. For clinical manifestations, bulbar symptoms and dyspnea were seen more frequently in patients with irMG. Serologic tests revealed that the frequency rates of anti-AchR antibody and anti-Musk antibody were significantly higher in idioMG group. Besides, the titer of anti-AChR antibodies was relatively low in patients with irMG compared to patients with idioMG. In irMG group, three patients were positive for anti-titin antibodies among nine patients tested (33.3%), which was not commonly seen in idioMG group. In irMG group, markedly elevated CK levels were observed with an average level of 5206.7 IU/L, which was scarcely found in idioMG group. In irMG group, 21 patients were diagnosed with myocarditis, while no patient had cardiac muscle involvement in idioMG group. Sixty one patients (96.8%) from irMG group required hospitalization after disease onset. Corticosteroids were used in more than 90% of patients for management for both irMG and idioMG. IVIg and PLEX were most commonly added to reduce the rapid progression of symptoms in irMG patients while infliximab and rituximab were used in 2 and 1 patients, respectively (33). For idioMG, PLEX was not commonly conducted in our center and we have no experience of using infliximab or rituximab. Unfavorable outcomes including death, intubation or tracheotomy was observed in 21 patients (35%) in irMG group, among which 14 patients (66.7%) died. Reasons of unfavorable outcomes include onset of a myasthenic crisis (13, 62%), infection or other complication (3, 14%) and cardiac incidence (5, 24%). Compared to irMG group, the incidence rate of unfavorable outcomes in idioMG group is relatively low. Discontinuation or withholding of ICI was recommended for

TABLE 1 | Demographic and baseline characteristics of patients.

	Total cohort, ($n = 63$), *N* (%)	PUMCH, ($n = 6$), *N* (%)
Indication for ICI		
Lung Carcinoma	10 (16.1)	
Melanoma	31 (50)	0 (0)
Urethral Carcinoma	13 (21.0)	0 (0)
Gynecological Carcinoma	1 (1.6)	1 (16.7)
Digestive system neoplasm	2 (3.2)	1 (16.7)
Others	3 (8.1)	1 (16.7)
Tumor Staging, *N* (%)	0	0
1		
2	2 (4.7)	1 (16.7)
3	7 (16.3)	0 (0)
4	34 (79.1)	5 (83.3)
Type of ICI applied, *N* (%)	45 (73.8)	6 (100)
PD-1		
CTLA-4	8 (13.1)	0 (0)
PD-1+CTLA-4	6 (9.8)	0 (0)
Others	2 (3.3)	0 (0)
Time from first ICI injection to symptom onset, median weeks (range)	5 (1–28)	5.5 (2–9)
Time from last ICI injection to symptom onset, median days (range)	10 (1–35)	13.5 (12–28)
Level of irAEs		
I	12 (19.4)	0 (0)
II	10 (16.1)	1 (16.7)
III	12 (19.4)	2 (33.3)
IV	28 (45.2)	3 (50.0)
Complicated with irAEs of other systems, *N* (%)	21 (33.3)	2 (33.3)
Myocarditis		
Elevated liver enzymes	7 (11.1)	2 (33.3)
Skin	4 (6.35)	1 (16.7)
Colitis or diarrhea	3 (4.76)	0 (0)
Hematological	2 (3.17)	1 (16.7)
Renal failure	1 (1.59)	0 (0)

TABLE 2 | irMG characteristics and treatment.

	irMG, ($n = 63$), *N* (%)	idioMG, ($n = 380$), *N* (%)	*p*
Median age, years (Range)	72 (44–86)	52 (2–84)	0.000
Male, *N* (%)	43 (69.4)	174 (45.8)	0.000
Past history of MG, *N* (%)	11 (19.0)	-	-
MGFA classification at first visit, *N* (%)			
I	16 (25.8)	122 (32.1)	0.001
II	10 (16.1)	133 (35.0)	
III	15 (24.2)	84 (22.1)	
IV	21 (34.9)	30 (7.9)	
V	0 (0.0)	11 (2.9)	
Clinical presentation, *N* (%)			
Ptosis	49 (89.1)	334 (88.2)	0.821
Diplopia	43 (78.2)	270 (71.3)	0.457
Dyspnea	30 (55.6)	41 (10.8)	0.001
Limb weakness	34 (63.0)	226 (59.6)	0.824
Dysphagia	32 (59.3)	69 (18.2)	0.002
QMGS rates at disease onset, (SD)	18.17 (11.4)	12.32 (8.2)	0.012
Antibody			
Positive anti-AchR Ab, *N* (%)	27 (56.3)	277 (73.0)	0.050
Average anti-AchR Ab, nmol/L, (SD)	4.5 (4.1)	7.8 (13.3)	0.081
Positive anti-Musk Ab, *N* (%)	1 (1.6)	27 (7.0)	0.020
Positive anti-Titin Ab, *N* (%)	3 (33.3)	NA	-
Complicated with myositis, *N* (%)	31 (63.3)	32 (8.4)	0.000
Complicated with myocarditis, *N* (%)	21 (41.2)	0 (0)	0.000
CK level, μmol (SD)	5206.7 (5048.3)	137.2 (125.1)	0.000
Treatment, *N* (%)			
IVIg	1 (1.9)	5 (1.3)	0.716
IVIg + corticosteroids	11 (20.4)	116 (30.5)	0.213
IVIg + corticosteroids + PLEX	18 (33.3)	10 (2.7)	0.002
Corticosteroids + PLEX	8 (14.8)	7 (1.8)	0.001
Corticosteroids	16 (29.6)	201 (52.9)	0.137
Infliximab	2 (3.2)	0 (0)	0.145
Rituximab	1 (1.6)	0 (0)	0.219
Outcome			
Tracheotomy, Intubation, or Death	21 (35.0)	23 (6.1)	0.001
Improvement	40 (65.0)	-	

61 patients (97%) in our cohort, while 2 patients continued ICI treatments with well-controlled MG symptoms (41).

Associations Between Demographic and Clinical Factors and irMG Outcome

Results of single variate binary logistic regression are shown in **Figure 2**. Application of cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and PD-1 inhibitors together was negatively related to an unfavorable outcome for ICI-related MG ($OR = 12.142$, $p = 0.050$). Evaluation parameters for MG severity, which included QMGS and MGFA classification at the first clinical visit, were indicative of disease outcome. A QMGS > 18.167 ($OR = 6.667$, $p = 0.035$) and MGFA classification IV ($OR = 1.036$, $p = 0.000$) were both related to unfavorable disease outcome. An overlap of myocarditis with irAEs in other systems was significantly associated with unfavorable irMG outcome. Although associated with myositis was not relevant to disease outcome, creatine kinase levels > 5,000 U/L were negatively related to disease outcome ($OR = 6.667$, $p = 0.023$). For treatment, we found that, compared to using steroids alone, administering IVIg, steroids plus PLEX, or IVIg plus steroids may be protective factors for irMG outcome. From the factors that were analyzed in the single variate binary logistic model, we included the type of ICI applied, MGFA classification, QMGS, overlap of myocarditis with other system irAEs, and treatments in the multivariate binary logistic regression model. The multivariate analysis (**Figure 3**) showed that, associated with myocarditis, QMGS ≥ 18.167 and MGFA classification IV were

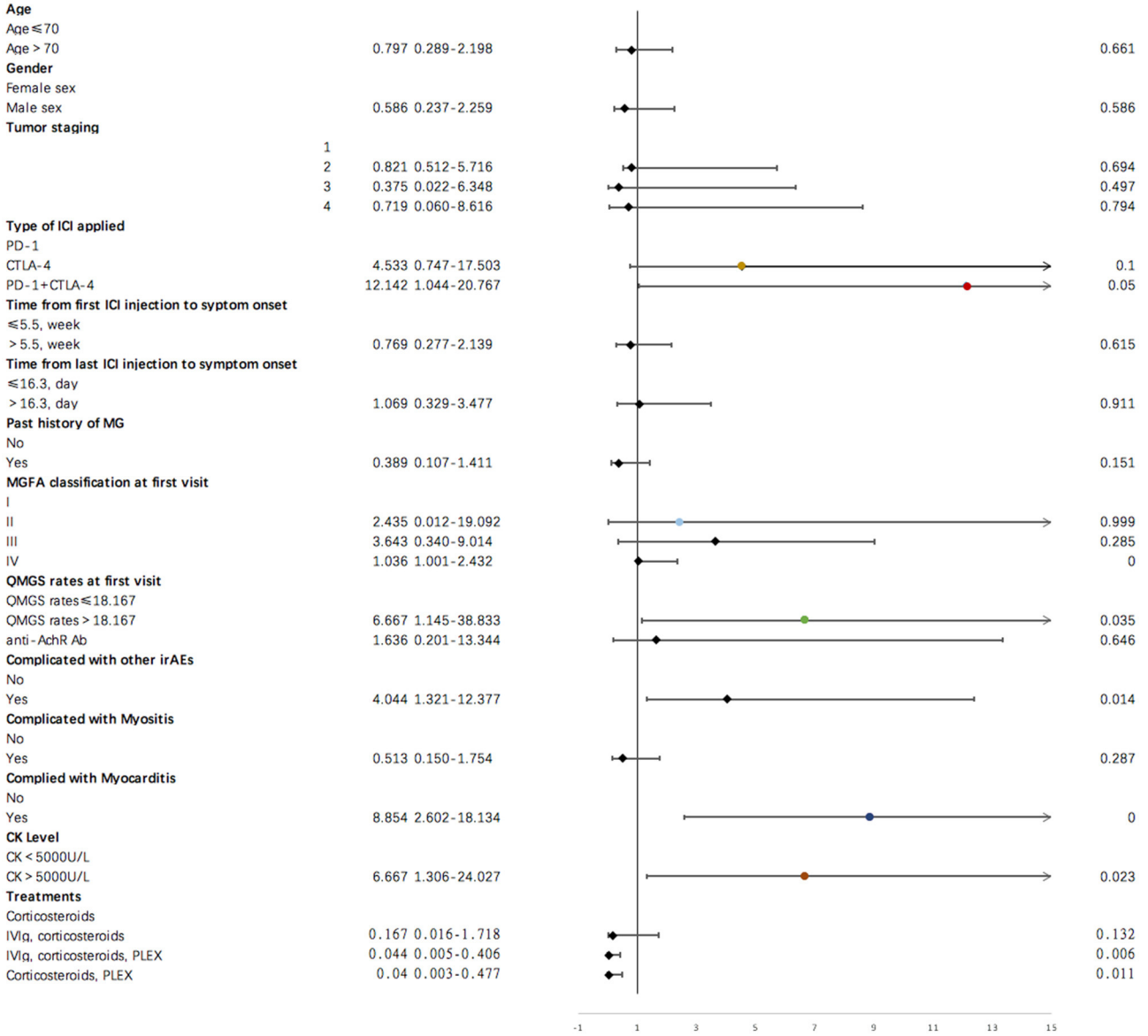


FIGURE 2 | Results of single variate binary logistic regression for demographic and clinical factors and irMG outcome.

negatively related to the outcome of irMG. Compared with corticosteroids alone, utilization of IVIg and PLEX may be a positive prognostic factor for irMG.

DISCUSSION

In this study, we report an extensive case series of ICI-related MG with detailed clinical features, treatments, and disease outcome. Our study innovatively identified several clinical factors that may be useful for predicting irMG prognosis.

Our findings support that irMG has several different clinical features compared with idioMG, which has also been proved by previous studies (10, 11). Demographically, the age at diagnosis of irMG was significantly greater than that of patients with idioMG. For clinical severity, the majority of idioMG patients fall within the MGFA classes I and II at the time of diagnosis and present with a slow clinical deterioration course (47), while the majority of irMG patients were categorized in MGFA classes III and IV at the first visit with a high QMGS rates. Serologically, the positive rate of anti-AchR antibody in idioMG patients has been reported to be around 70–80% (49–51), which is statistically higher than the positive rate of irMG group. For anti-MUSK antibody, positive rate in idioMG patients is ∼5–10% (51), while in irMG group, positive rate was only 1.6%. This finding shows that the prevalence of seronegative patients in irMG was higher than that in classical MG, which has been

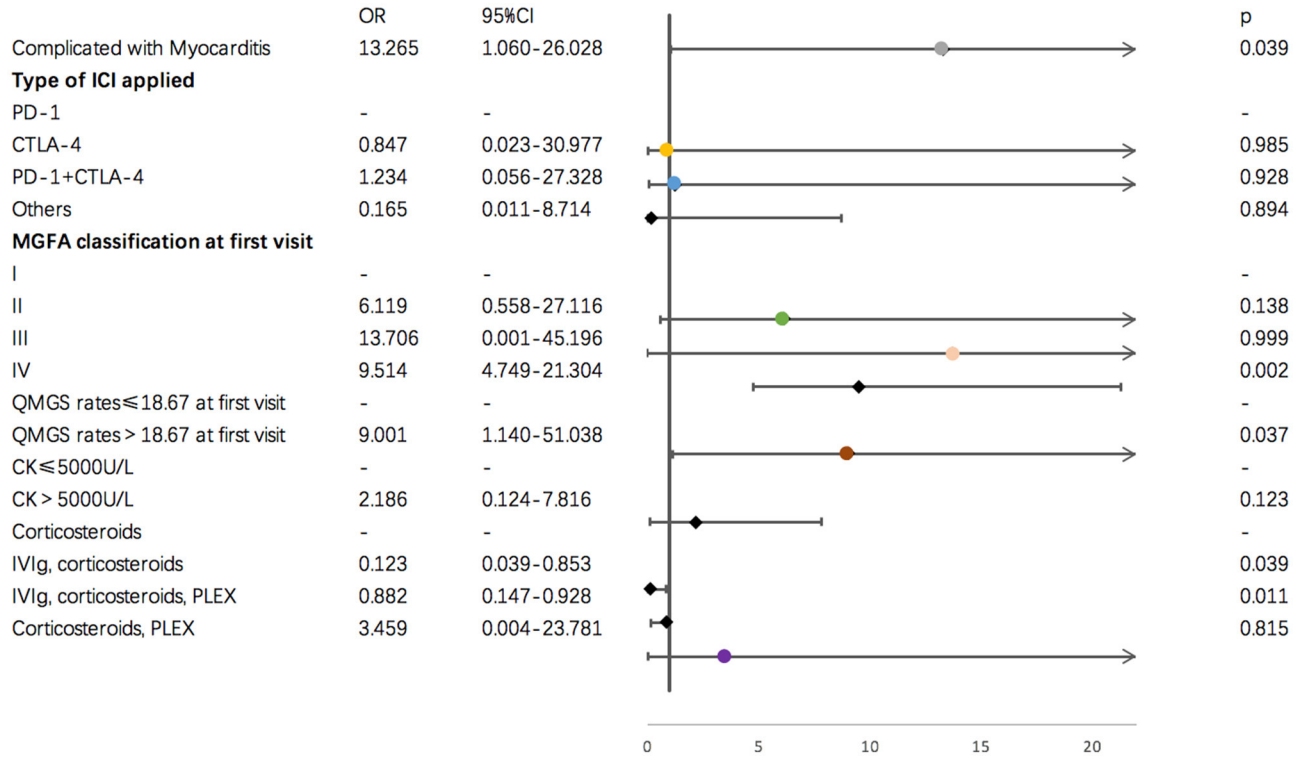


FIGURE 3 | Results of multi-variate binary logistic regression for demographic and clinical factors and irMG outcome.

previously proved by other studies (7, 41, 52). The demographic and serological differences could be caused by the bias due to that case reports and case series tend to report irMG patients with more severe clinical manifestations, still the differences that we observed indicated that irMG and idioMG are clinically distinct disease entity.

We also observed that irMG were more likely to be associated with myositis or myocarditis, which has been described in only 0.9% of patients with idioMG (6, 7, 53). Some researchers believe that the elevation of serum CK in patients with irMG reflects inflammatory involvement of skeletal muscles rather than rhabdomyolysis (7). Other investigators have hypothesized that myositis is the main clinical manifestation of irMG patients, whereas a positive antibody result is a marker of activated autoimmunity (23, 54). High association rate of myocarditis in irMG group is noticeable. Although the mechanism of this phenomenon is now still not well-established, molecular mimicry and the critical role of PD-1 signaling pathways in regulating autoimmune responses myocardium might be responsible (19).

It is important for physicians to identify factors that might be indicative of disease outcome. We found that higher MGFA classification and a higher QMGS at the first visit were predictive for an unfavorable disease outcome. Both MGFA classification and QMGS are parameters for prognosis prediction and severity evaluation in idioMG patients, and our study supported that the utilization of these measures is valid in irMG patients. It has been demonstrated that associated with myositis may increase muscle weakness in patients with irMG (7, 51, 53), suggesting that a substantial proportion of an irMG patient's clinical symptoms is associated with the accompanying myositis. Although associating with myositis was not related to disease outcome in our analysis, still in single variate analysis, we have found that CK>5,000 U/L was a negative prognosis factor. Thus, awareness of early recognition of muscle involvement in possible irMG patients is important. In this regard, serum CK tests before and after treatment with irMG is required. Association with myocarditis has been reported to be negatively related to irAEs outcome (22, 47, 54). Because it is not uncommon for irMG patients to have myocarditis [39.7% in our cohort, 20%−40% in published series cases (4, 55, 56)], we believe that particular attention including ECG, echocardiography and serum troponin tests should be conducted for myocarditis identification to allow timely multidisciplinary management.

Our data suggest that patients who received IVIg or PLEX experienced improved irMG outcomes compared with those who received steroids alone. Although corticosteroids are recommended as a first-line treatment for irMG (2, 57), the use of steroids as a sole first-line therapy may not be ideal because steroid use itself can cause an acute exacerbation of MG symptoms (58). Although the worsening in symptoms has been described as transient (59), the use of steroids alone in irMG may be associated with a poorer prognosis because these patients may not be able to survive a transient worsening of symptoms considering their older age and advanced stage of malignancy

(41). Apart from this, the role of steroids in controlling immune dysregulation in irMG patients might be limited by the constant presence of the circulating ICIs as the original trigger of irAEs. Since IVIg and PLEX could accelerate the process of ICIs mAbs elimination, they could mediate a faster improvement of symptoms (41). From our clinical experience and analysis, the use of IVIg and PLEX together with corticosteroids has led to favorable outcomes in irMG patients, which has been demonstrated in other studies as well (3, 19, 41, 60). However, for irMG initial treatment, no consistent conclusion could be drawn from the big variety of published reports. Given the small number of patients and the retrospective nature of our study, we think further researches for irMG treatment is highly required. Besides, physicians should be aware of early treatments of vital organ dysfunction. Timely intubation for respiratory failure, pacemaker implantation for fatal arrhythmia, and vasopressor and even extracorporeal membrane oxygenation for cardiogenic shock should be considered if clinically needed and available.

Our study has some limitations. Although it is the first study that identifies possible factors responsible for irMG outcome, the relatively small sample size and retrospective nature of the study design limit the reliability of our study results. Because of the variability in the data available from case reports or case series, there were missing data regarding clinical features, hospital course, and outcomes of some patients, which subjected our results to reporting bias. Besides, the information obtained from the collected case reports represents only a small fraction of the actual number of cases worldwide. Nevertheless, our study enhances the understanding of irMG clinical manifestations and factors involved in irMG prognosis.

With the boost of ICIs utilization and awareness of the disease, we believe that the number of patients with irMG is poised to rapid increase. Additional therapeutic studies concerning irMG in the future are needed to decrease the irAE-related mortality and increase the safety of immune therapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Peking Union Medical College Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JS and YG contributed to the conception and design of the study. JS, YT, YH, KL, and JY collected the clinical data. JS and YH contributed to the potential article identifications and article quality evaluation. JS wrote the manuscript. YG and LZ edited the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by the National Clinical Cohort Study of Rare Diseases for Key R&D Program (Grant Number: 2016YFC091501) and Beijing Municipal Science & Technology Commission Capital Characteristic Clinic Project Z181100001718145.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.858628/full#supplementary-material

REFERENCES

1. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science (New York, NY).* (2015) 348:56–61. doi: 10.1126/science.aaa8172
2. Shi J, Niu J, Shen D, Liu M, Tan Y, Li Y, et al. Clinical diagnosis and treatment recommendations for immune checkpoint inhibitor-related adverse reactions in the nervous system. *Thorac Cancer.* (2020) 11:481–7. doi: 10.1111/1759-7714.13266
3. Diamanti L, Picca A, Bini P, Gastaldi M, Alfonsi E, Pichiecchio A, et al. Characterization and management of neurological adverse events during immune-checkpoint inhibitors treatment: an Italian multicentric experience. *Neurol Sci.* (2021) 43:2031–41. doi: 10.1007/s10072-021-05561-z
4. Johansen A, Christensen SJ, Scheie D, Højgaard JLS, Kondziella D. Neuromuscular adverse events associated with anti-PD-1 monoclonal antibodies: systematic review. *Neurology.* (2019) 92:663–74. doi: 10.1212/WNL.0000000000007235
5. Bolz S, Ramakrishnan T, Fleischer M, Livingstone E, Stolte B, Thimm A, et al. Detect it so you can treat it: a case series and proposed checklist to detect neurotoxicity in checkpoint therapy. *eNeurologicalSci.* (2021) 22:100324. doi: 10.1016/j.ensci.2021.100324
6. Drachman DB. Myasthenia gravis. *N Engl J Med.* (1994) 330:1797–810. doi: 10.1056/NEJM199406233302507
7. Suzuki S, Ishikawa N, Konoeda F, Seki N, Fukushima S, Takahashi K, et al. Nivolumab-related myasthenia gravis with myositis and myocarditis in Japan. *Neurology.* (2017) 89:1127–34. doi: 10.1212/WNL.0000000000004359
8. Makarious D, Horwood K, Coward JIG. Myasthenia gravis: an emerging toxicity of immune checkpoint inhibitors. *Eur J Cancer (Oxford, England: 1990).* (2017) 82:128–36. doi: 10.1016/j.ejca.2017.05.041
9. Khokher W, Bhuta S, Kesireddy N, Abuhelwa Z, Mhanna M, Iftikhar S, et al. Refractory nivolumab-induced myasthenia gravis treated with abatacept. *Am J Ther.* (2021). [Epub ahead of print]. doi: 10.1097/mjt.0000000000001405
10. Alnahhas I, Wong J. A case of new-onset antibody-positive myasthenia gravis in a patient treated with pembrolizumab for melanoma. *Muscle Nerve.* (2017) 55:E25–e6. doi: 10.1002/mus.25496
11. Algaeed M, Mukharesh L, Heinzelmann M, Kaminski HJ. Pearls & Oy-sters: pembrolizumab-induced myasthenia gravis. *Neurology.* (2018) 91:e1365–e7. doi: 10.1212/WNL.0000000000006278
12. Fukasawa Y, Sasaki K, Natsume M, Nakashima M, Ota S, Watanabe K, et al. Nivolumab-induced myocarditis concomitant with myasthenia gravis. *Case Rep Oncol.* (2017) 10:809–12. doi: 10.1159/000479958
13. Johnson DB, Saranga-Perry V, Lavin PJ, Burnette WB, Clark SW, Uskavitch DR, et al. Myasthenia gravis induced by ipilimumab in patients with metastatic melanoma. *J Clin Oncol.* (2015) 33:e122–4. doi: 10.1200/JCO.2013.51.1683

14. Liu Q, Ayyappan S, Broad A, Narita A. Pembrolizumab-associated ocular myasthenia gravis. *Clin Experiment Ophthalmol.* (2019) 47:796–8. doi: 10.1111/ceo.13499
15. Liao B, Shroff S, Kamiya-Matsuoka C, Tummala S. Atypical neurological complications of ipilimumab therapy in patients with metastatic melanoma. *Neuro Oncol.* (2014) 16:589–93. doi: 10.1093/neuonc/nou001
16. Mathews EP, Romito JW. Management of immune checkpoint inhibitor-related acute hypoxic neuromuscular respiratory failure using high-flow nasal cannula. *Proceedings (Baylor University Medical Center).* (2020) 33:407–8. doi: 10.1080/08998280.2020.1744793
17. Montes V, Sousa S, Pita F, Guerreiro R, Carmona C. Myasthenia gravis induced by ipilimumab in a patient with metastatic melanoma. *Front Neurol.* (2018) 9:150. doi: 10.3389/fneur.2018.00150
18. Mehta JJ, Maloney E, Srinivasan S, Seitz P, Cannon M. Myasthenia gravis induced by nivolumab: a case report. *Cureus.* (2017) 9:e1702. doi: 10.7759/cureus.1702
19. Pathak R, Katel A, Massarelli E, Villaflor VM, Sun V, Salgia R. Immune checkpoint inhibitor-induced myocarditis with myositis/myasthenia gravis overlap syndrome: a systematic review of cases. *Oncologist.* (2021) 26:1052–61. doi: 10.1002/onco.13931
20. Onda A, Miyagawa S, Takahashi N, Gochi M, Takagi M, Nishino I, et al. Pembrolizumab-induced ocular myasthenia gravis with anti-titin antibody and necrotizing myopathy. *Intern Med (Tokyo, Japan).* (2019) 58:1635–8. doi: 10.2169/internalmedicine.1956-18
21. Phadke SD, Ghabour R, Swick BL, Swenson A, Milhem M, Zakharia Y. Pembrolizumab therapy triggering an exacerbation of preexisting autoimmune disease: a report of 2 patient cases. *J Investig Med High Impact Case Rep.* (2016) 4:2324709616674316. doi: 10.1177/2324709616674316
22. Salim A, Tapia Rico G, Shaikh A, Brown MP. A systematic review of immune checkpoint inhibitor-related neurological adverse events and association with anti-neuronal autoantibodies. *Expert Opin Biol Ther.* (2021) 21:1237–51. doi: 10.1080/14712598.2021.1897101
23. Shelly S, Triplett JD, Pinto MV, Milone M, Diehn FE, Zekeridou A, et al. Immune checkpoint inhibitor-associated myopathy: a clinicoseropathologically distinct myopathy. *Brain Commun.* (2020) 2:fcaa181. doi: 10.1093/braincomms/fcaa181
24. Takai M, Kato D, Iinuma K, Maekawa YM, Nakane K, Tsuchiya T, et al. Simultaneous pembrolizumab-induced myasthenia gravis and myocarditis in a patient with metastatic bladder cancer: a case report. *Urol Case Rep.* (2020) 31:101145. doi: 10.1016/j.eucr.2020.101145
25. Takizawa T, Kojima M, Suzuki S, Osada T, Kitagawa S, Nakahara J, et al. New onset of myasthenia gravis after intravesical Bacillus Calmette-Guerin: a case report and literature review. *Medicine.* (2017) 96:e8757. doi: 10.1097/MD.0000000000008757
26. Todo M, Kaneko G, Shirotake S, Shimada Y, Nakano S, Okabe T, et al. Pembrolizumab-induced myasthenia gravis with myositis and presumable myocarditis in a patient with bladder cancer. *IJU Case Rep.* (2020) 3:17–20. doi: 10.1002/iju5.12128
27. Tozuka T, Sugano T, Noro R, Takano N, Hisakane K, Takahashi S, et al. Pembrolizumab-induced agranulocytosis in a pulmonary pleomorphic carcinoma patient who developed interstitial lung disease and ocular myasthenia gravis. *Oxford Med Case Rep.* (2018) 2018:omy094. doi: 10.1093/omcr/omy094
28. Tomisaki I. Editorial comment to pembrolizumab-induced myasthenia gravis with myositis and presumable myocarditis in a patient with bladder cancer. *IJU Case Rep.* (2020) 3:20. doi: 10.1002/iju5.12132
29. Wilson R, Menassa DA, Davies AJ, Michael S, Hester J, Kuker W, et al. Seronegative antibody-mediated neurology after immune checkpoint inhibitors. *Ann Clin Transl Neurol.* (2018) 5:640–5. doi: 10.1002/acn3.547
30. Zhu J, Li Y. Myasthenia gravis exacerbation associated with pembrolizumab. *Muscle Nerve.* (2016) 54:506–7. doi: 10.1002/mus.25055
31. Dang T, Macwan S, Dasanu CA. Late-onset double-seronegative myasthenia gravis syndrome and myasthenic crisis due to nivolumab use for Hodgkin's lymphoma. *J Oncol Pharm Pract.* (2020) 2020:1078155220976797. doi: 10.1177/1078155220976797
32. March KL, Samarin MJ, Sodhi A, Owens RE. Pembrolizumab-induced myasthenia gravis: a fatal case report. *J Oncol Pharm Pract.* (2018) 24:146–9. doi: 10.1177/1078155216687389
33. Rajendram P, Torbic H, Duggal A, Campbell J, Hovden M, Dhawan V, et al. Critically ill patients with severe immune checkpoint inhibitor related neurotoxicity: a multi-center case series. *J Crit Care.* (2021) 65:126–32. doi: 10.1016/j.jcrc.2021.05.020
34. Puwanant A, Isfort M, Lacomis D, Živković SA. Clinical spectrum of neuromuscular complications after immune checkpoint inhibition. *Neuromuscul Disord.* (2019) 29:127–33. doi: 10.1016/j.nmd.2018.11.012
35. Shirai T, Kiniwa Y, Sato R, Sano T, Nakamura K, Mikoshiba Y, et al. Presence of antibodies to striated muscle and acetylcholine receptor in association with occurrence of myasthenia gravis with myositis and myocarditis in a patient with melanoma treated with an anti-programmed death 1 antibody. *Eur J Cancer (Oxford, England: 1990).* (2019) 106:193–5. doi: 10.1016/j.ejca.2018.10.025
36. Yang Y, Xu L, Wang D, Hui B, Li X, Zhou Y, et al. Anti-PD-1 and regorafenib induce severe multisystem adverse events in microsatellite stability metastatic colorectal cancer: a case report. *Immunotherapy.* (2021) 13:1317–23. doi: 10.2217/imt-2020-0327
37. Ziobro AS, LaPlante RL, DeMari SR, Clark LM, Kingsley DJ, Smith AJ. Myasthenia gravis associated with Programmed Death-1 (PD-1) receptor inhibitor pembrolizumab: a 40-day case report. *J Pharm Pract.* (2021) 34:166–70. doi: 10.1177/0897190020970750
38. Jeyakumar N, Etchegaray M, Henry J, Lelenwa L, Zhao B, Segura A, et al. The terrible triad of checkpoint inhibition: a case report of myasthenia gravis, myocarditis, and myositis induced by cemiplimab in a patient with metastatic cutaneous squamous cell carcinoma. *Case Rep Immunol.* (2020) 2020:5126717. doi: 10.1155/2020/5126717
39. Hayakawa N, Kikuchi E, Suzuki S, Oya M. Myasthenia gravis with myositis induced by pembrolizumab therapy in a patient with metastatic urothelial carcinoma. *Int Cancer Conf J.* (2020) 9:123–6. doi: 10.1007/s13691-020-00408-4
40. Botta C, Agostino RM, Dattola V, Cianci V, Calandruccio ND, Bianco G, et al. Myositis/myasthenia after pembrolizumab in a bladder cancer patient with an autoimmunity-associated HLA: immune-biological evaluation and case report. *Int J Mol Sci.* (2021) 22:6246. doi: 10.3390/ijms22126246
41. Safa H, Johnson DH, Trinh VA, Rodgers TE, Lin H, Suarez-Almazor ME, et al. Immune checkpoint inhibitor related myasthenia gravis: single center experience and systematic review of the literature. *J Immunother Cancer.* (2019) 7:319. doi: 10.1186/s40425-019-0774-y
42. Hibino M, Maeda K, Horiuchi S, Fukuda M, Kondo T. Pembrolizumab-induced myasthenia gravis with myositis in a patient with lung cancer. *Respirol Case Rep.* (2018) 6:e00355. doi: 10.1002/rcr2.355
43. Lau KH, Kumar A, Yang IH, Nowak RJ. Exacerbation of myasthenia gravis in a patient with melanoma treated with pembrolizumab. *Muscle Nerve.* (2016) 54:157–61. doi: 10.1002/mus.25141
44. Nguyen BH, Kuo J, Budiman A, Christie H, Ali S. Two cases of clinical myasthenia gravis associated with pembrolizumab use in responding melanoma patients. *Melanoma Res.* (2017) 27:152–4. doi: 10.1097/CMR.0000000000000310
45. Hajihossainlou B, Vasileva A, Manthri S, Chakraborty K. Myasthenia gravis induced or exacerbated by immune checkpoint inhibitors: a rising concern. *BMJ Case Rep.* (2021) 14:e243764. doi: 10.1136/bcr-2021-243764
46. Giglio D, Berntsson H, Fred Å, Ny L. Immune Checkpoint Inhibitor-Induced Polymyositis and Myasthenia Gravis with Fatal Outcome. *Case Rep Oncol.* (2020) 13:1252–7. doi: 10.1159/000510740
47. Melzer N, Ruck T, Fuhr P, Gold R, Hohlfeld R, Marx A, et al. Clinical features, pathogenesis, and treatment of myasthenia gravis: a supplement to the Guidelines of the German Neurological Society. *J Neurol.* (2016) 263:1473–94. doi: 10.1007/s00415-016-8045-z
48. Derle E, Benli S. Ipilimumab treatment associated with myasthenic crises and unfavorable disease course. *Neurol Sci.* (2018) 39:1773–4. doi: 10.1007/s10072-018-3471-6
49. Dresser L, Wlodarski R, Rezania K, Soliven B. Myasthenia gravis: epidemiology, pathophysiology and clinical manifestations. *J Clin Med.* (2021) 10:2235. doi: 10.3390/jcm10112235
50. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol.* (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3

51. Andersen JB, Gilhus NE, Sanders DB. Factors affecting outcome in myasthenia gravis. *Muscle Nerve.* (2016) 54:1041–9. doi: 10.1002/mus.25205
52. Huang YT, Chen YP, Lin WC, Su WC, Sun YT. Immune checkpoint inhibitor-induced myasthenia gravis. *Front Neurol.* (2020) 11:634. doi: 10.3389/fneur.2020.00634
53. Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. *Muscle Nerve.* (2008) 37:141–9. doi: 10.1002/mus.20950
54. Hamada N, Maeda A, Takase-Minegishi K, Kirino Y, Sugiyama Y, Namkoong H, et al. Incidence and distinct features of immune checkpoint inhibitor-related myositis from idiopathic inflammatory myositis: a single-center experience with systematic literature review and meta-analysis. *Front Immunol.* (2021) 12:803410. doi: 10.3389/fimmu.2021.803410
55. Moslehi JJ, Salem JE, Sosman JA, Lebrun-Vignes B, Johnson DB. Increased reporting of fatal immune checkpoint inhibitor-associated myocarditis. *Lancet (London, England).* (2018) 391:933. doi: 10.1016/S0140-6736(18)30533-6
56. Touat M, Maisonobe T, Knauss S, Ben Hadj Salem O, Hervier B, Auré K, et al. Immune checkpoint inhibitor-related myositis and myocarditis in patients with cancer. *Neurology.* (2018) 91:e985–e94. doi: 10.1212/WNL.0000000000006124
57. Puzanov I, Diab A, Abdallah K, Bingham CO. 3rd, Brogdon C, Dadu R, et al. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J Immunother Cancer.* (2017) 5:95. doi: 10.1186/s40425-017-0300-z
58. Miller RG, Milner-Brown HS, Mirka A. Prednisone-induced worsening of neuromuscular function in myasthenia gravis. *Neurology.* (1986) 36:729–32. doi: 10.1212/WNL.36.5.729
59. Bae JS, Go SM, Kim BJ. Clinical predictors of steroid-induced exacerbation in myasthenia gravis. *J Clin Neurosci.* (2006) 13:1006–10. doi: 10.1016/j.jocn.2005.12.041
60. Sutaria R, Patel P, Danve A. Autoimmune myositis and myasthenia gravis resulting from a combination therapy with nivolumab and ipilimumab for metastatic melanoma. *Eur J Rheumatol.* (2019) 6:153–4. doi: 10.5152/eurjrheum.2019.18159

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 08 April 2022
doi: 10.3389/fneur.2022.879261

Clinical Features of Myasthenia Gravis With Antibodies to MuSK Based on Age at Onset: A Multicenter Retrospective Study in China

Yufan Zhou[1,2,3†], *Jialin Chen*[4†], *Zunbo Li*[5], *Song Tan*[6,7], *Chong Yan*[1,2,3], *Sushan Luo*[1,2,3], *Lei Zhou*[1,2,3], *Jie Song*[1,2,3], *Xiao Huan*[1,2,3], *Ying Wang*[8], *Chongbo Zhao*[1,2,3], *Wenshuang Zeng*[9*] and *Jianying Xi*[1,2,3*]

[1] Department of Neurology, Huashan Hospital, Fudan University, Shanghai, China, [2] Huashan Rare Disease Center, Huashan Hospital, Fudan University, Shanghai, China, [3] National Center for Neurological Diseases, Shanghai, China, [4] Department of Neurology, Fujian Medical University Union Hospital, Fuzhou, China, [5] Department of Neurology, Xi'an Gaoxin Hospital, Xi'an, China, [6] Department of Neurology, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, China, [7] Chinese Academy of Sciences Sichuan Translational Medicine Research Hospital, Chengdu, China, [8] Department of Pharmacy, Huashan Hospital, Fudan University, Shanghai, China, [9] Department of Neurology, The University of Hong Kong-Shenzhen Hospital, Shenzhen, China

OPEN ACCESS

Edited by:
Nils Erik Gilhus,
University of Bergen, Norway

Reviewed by:
Yuwei Da,
Capital Medical University, China
Timothy John Day,
Royal Melbourne Hospital, Australia

***Correspondence:**
Jianying Xi
xijianying@fudan.edu.cn
Wenshuang Zeng
zengws@hku-szh.org

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: 19 February 2022
Accepted: 18 March 2022
Published: 08 April 2022

Citation:
Zhou Y, Chen J, Li Z, Tan S, Yan C, Luo S, Zhou L, Song J, Huan X, Wang Y, Zhao C, Zeng W and Xi J (2022) Clinical Features of Myasthenia Gravis With Antibodies to MuSK Based on Age at Onset: A Multicenter Retrospective Study in China. Front. Neurol. 13:879261. doi: 10.3389/fneur.2022.879261

Introduction: Antibodies to MuSK identify a rare subtype of myasthenia gravis (MuSK-MG). In western countries, the onset age of MuSK-MG peaks in the late 30's while it is unknown in Chinese population.

Methods: In this retrospective multicenter study, we screened 69 MuSK-MG patients from 2042 MG patients in five tertiary referral centers in China from October 2016 to October 2021 and summarized the clinical features and treatment outcomes. Then we subgrouped the patients into early-onset (<50 years old), late-onset (50–64 years old), and very-late-onset (≥65 years old) MG and compared the differences in weakness distribution, disease progression and treatment outcomes among three subgroups.

Results: The patients with MuSK-MG were female-dominant (55/69) and their mean age at onset was 44.70 ± 15.84 years old, with a broad range of 17–81 years old. At disease onset, 29/69 patients were classified as MGFA Type IIb and the frequency of bulbar and extraocular involvement was 53.6 and 69.6%, respectively. There was no difference in weakness distribution. Compared with early-onset MuSK-MG, very-late-onset patients had a higher proportion of limb muscle involvement (12/15 vs.16/40, $p = 0.022$) 3 months after onset. Six months after onset, more patients with bulbar (14/15 vs. 26/39, $p = 0.044$) and respiratory involvement (6/15 vs. 0/13, $p = 0.013$) were seen in very-late-onset than in late-onset subgroup. The very-late-onset subgroup had the highest frequency of limb weakness (86.7%, $p < 0.001$). One year after onset, very-late-onset patients demonstrated a higher frequency of respiratory involvement than early-onset patients (4/12 vs. 2/35, $p = 0.036$). 39/64 patients reached MSE. Among 46 patients who received rituximab, very-late-onset patients started earlier than late-onset patients [6 (5.5–7.5) vs. 18 (12–65) months, $p = 0.039$], but no difference in the time and rate to achieving MSE was identified.

Conclusion: MuSK-MG patients usually manifested as acute onset and predominant bulbar and respiratory involvement with female dominance. Very-late-onset patients displayed an early involvement of limb, bulbar and respiratory muscles in the disease course, which might prompt their earlier use of rituximab. The majority MuSK-MG patients can benefit from rituximab treatment regardless of age at onset.

Keywords: myasthenia gravis, muscle-specific tyrosine kinase, clinical features, weakness distribution, age at onset

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune neuromuscular disorder characterized by circulating autoantibodies against functionally important components of the postsynaptic membrane, including acetylcholine receptor (AChR) and muscle-specific tyrosine kinase (MuSK). MuSK is essentially a neuromuscular junction protein, which is closely related to the assembly of AChR. MG with antibodies against MuSK (MuSK-MG) is a rare subtype and it is found that only 0–6% in patients with MG (1–4). MuSK-MG is phenotypically different from anti-AChR antibody-positive MG (AChR-MG) by prominent involvement of bulbar muscles and rapid progression to myasthenia crisis. Furthermore, they show a poor response to acetylcholinesterase inhibitors (ACEI), intravenous immunoglobulin (IVIg), standard immunosuppressant therapies, and thymectomy (5–8).

AChR-MG has been divided into distinct groups according to age at onset and the pathology of the thymus (9). Several studies have suggested the clinical differences among age subgroups, including distribution of muscle weakness, disease severity and response to immunotherapy (10–14). In MuSK-MG, it was reported that the majority were women aged between 30 and 40 years old (7, 15). However, recent data from our MG cohort indicated that the incidence of MuSK-MG in old patient has been increasing mainly due to the aging of the general population. MuSK-MG is still considered as a rare disease entity and no studies compared the clinical features among different age subgroups. We thus summarized the clinical features, longitudinal courses, and treatment outcome in a cohort of 69 MuSK-MG patients gathered from five tertiary referral centers in China and compared the difference among early, late and very-late-onset (16) subgroups.

METHODS

Study Design and Patient Recruitment

In this observational retrospective multicenter study, we selected MuSK-MG patients in five tertiary referral centers from October 2016 to October 2021. These tertiary referral centers included Huashan Hospital, Fudan University in Shanghai, Fujian Medical University Union Hospital in Fujian Province, Xi'an Gaoxin Hospital in Shanxi Province, Sichuan Provincial People's Hospital in Sichuan Province, and the University of Hong Kong-Shenzhen Hospital in Guangdong Province (**Figure 1**). The onset age of all patients was older than 16 years. We classified the patients into the following subgroups: early-onset (patients with age at onset younger than 50 years old), late-onset (patients with age at onset 50–64 years old), and very-late-onset (patients with age at onset no $\geq$65 years old) subgroups. Written informed consent was granted by each patient and the study was approved by the Institutional Review Board of Huashan Hospital, Fudan University.

Evaluation and Collection

We evaluated the following variables: demographic characteristics (gender, age at onset); diagnostic delay, defined as the time from the date of onset to diagnosis; Myasthenia Gravis Foundation of America (MGFA) classification (17) at disease onset and at maximal worsening; the time from onset to maximal worsening; distribution of muscle weakness at onset, 3 months, 6 months, and 1 year after onset, respectively; disease progression, defined as a new muscle group involvement 1 month after onset, including progression from ocular to bulbar muscles, from ocular to bulbar and limb muscles, from limb to bulbar muscles, from bulbar to limb muscles, and from any to respiratory muscles; myasthenic crisis (MC), defined as an event that requires mechanical ventilation because of severe involvement of respiratory muscles (18); the time from onset to MC; immunotherapy, including steroids, IVIg, plasma exchange (PE), conventional non-steroid immunosuppressant (azathioprine, tacrolimus, mycophenolate mofetil, cyclophosphamide, cyclosporine) and rituximab; refractory MuSK-MG, defined as MGFA postintervention status (MGFA-PIS) (19) unchanged or worse after steroids and at least one other non-steroid immunosuppressant; comorbid disease, including thyroid abnormalities, urticaria, eczema, hypertension, diabetes mellitus, etc.; minimal symptom expression (MSE), defined as the MG-Related Activities of Daily Living score (MG-ADL) is 0 or 1 score (20); follow-up period, defined as the time from disease onset to the last visit; the positive rate of repetitive nerve stimulation (RNS) at 3 Hz at the time of initial diagnosis.

Abbreviations: Ab, Autoantibody; ACEI, Acetylcholinesterase inhibitors; AChR, Acetylcholine receptor; ANA, Antinuclear-antibody; IQR, Interquartile range; IVIg, Intravenous immunoglobulin; MC, Myasthenic crisis; MG, Myasthenia Gravis; MG-ADL, MG-Related Activities of Daily Living score; MGFA, Myasthenia Gravis Foundation of America; MGFA-PIS, MGFA postintervention status; MSE, Minimal symptom expression; MuSK, Muscle specific tyrosine kinase; PE, Plasma exchange; RNS, Repetitive nerve stimulation.

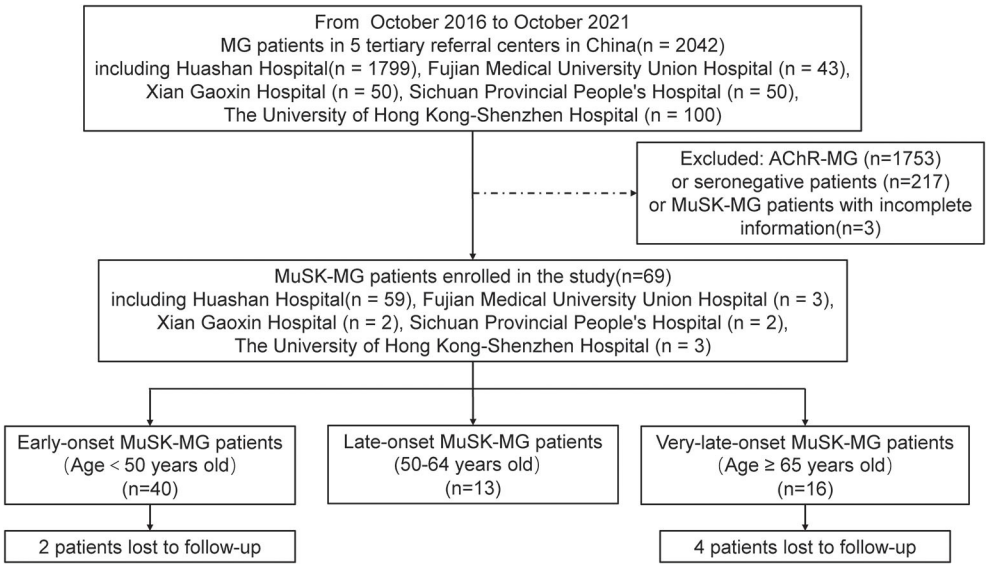


FIGURE 1 | Flowchart for the process of patient inclusion.

Statistical Analysis

Continuous variables that followed a normal distribution are presented as the mean ± standard deviation. Non-normally distributed data are presented as the median (interquartile range, IQR). Categorical variables are expressed as frequencies (percentages). Missing data were dropped as they were <20% of the sample for the relevant variables. Differences between subgroups were evaluated using the chi-square test and Fisher exact test for categorical variables, and the Kruskal Wallis H test to compare quantitative variables. *P*-values were adjusted by the Berforroni method. Kaplan–Meier curves and log-rank tests were used to compare the time and rate to achieve MSE status after rituximab treatment among three subgroups. A significant difference was defined as $p < 0.05$. Statistically significant variables were analyzed within each age group. Data analysis was carried out using IBM SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). Diagram generation were all conducted in R version 3.63 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Clinical Features of MuSK-MG Cohort

In our MG cohort of 2,042 patients from five tertiary referral centers, about 3.5% (72) patients are MuSK-MG, comprising 24.9% (72/289) AChR-negative patients. We finally included 69 MuSK-MG in this study. Demographics and clinical features were summarized in **Table 1**. The patients showed a female predominance (55/69), and the mean age at onset was 44.70 ± 15.84 years old, with a broad range of 17–81 years old (**Figure 2**). The median diagnostic delay was 5 [(IQR) 1–8.5] months and the median disease course was 34 [(IQR) 16.5–56] months. At disease onset, most patients (29/69) were classified as MGFA IIb (**Figure 2**) and the frequencies of bulbar, limb, and extraocular muscle involvement were 53.6, 29.0, and 69.6%, respectively. Fluctuating weakness was reported in 69.6% (48/69) patients and 80.4% (41/51) showed a positive neostigmine test. Regarding electrophysiological examination, 63 patients underwent repetitive nerve stimulation (RNS) test and 71.4% showed an abnormal decrease at low-frequency stimulation (3 Hz) and the muscle with the highest sensitivity was orbicularis oculi (53.6%). Abd Pollicis Brevis, frontalis, deltoid and trapezius showed a relatively low positive rate of 12.5, 16.1, 20, and 21.8%, respectively (**Supplementary Table 1**). Nineteen patients combined with other chronic diseases, including eight with hypertension, six with diabetes mellitus, five with hyperlipidemia, five with hepatitis B, two with latent tuberculosis, and one with breast cancer but no checkpoint inhibitor usage. Coexisted other autoimmune diseases were reported in 18 patients, including eight with thyroid abnormalities, three with urticaria, one with eczema, and eleven with positive antinuclear-antibody (ANA).

Forty-six out of 69 patients displayed disease progression, most of which (31/466) occurred in the first 6 months (**Figure 2**). Myasthenic crisis (MC) occurred in 31.9% (22/69) patients, and 50% (11/22) showed MC within 6 months after onset. The median duration from onset to disease progression was 4.5 [(IQR) 2–9.25] months and from onset to MC was 7 [(IQR) 2.75–13] months. Longitudinal disease progression and weakness distribution were shown in **Figure 3** and **Table 2**. Eighteen out of 69 patients showed a pure extraocular and 11/69 showed a pure bulbar phenotype at the onset. Two (2/18) patients remained pure extraocular involvement 1 year after onset and the two patients progressed to generalized MG 15 months (bulbar and limb involvement) and 48 months (bulbar involvement) after onset, respectively. Four patients remained pure bulbar involvement 1 year after onset and no further progression was observed. The proportion of respiratory involvement (11/67) was

TABLE 1 | Clinical features of early-onset, late-onset, and very-late-onset MuSK-myasthenia gravis (MuSK-MG).

Variables	Total $N = 69$	Early-onset $N = 40$	Late-onset $N = 13$	Very-late-onset $N = 16$	*P*-value
Female: male	55:14	34:6	9:4	12:4	0.384
Age at onset (years old) (mean ± SD)	44.70 ± 15.84	33.43 ± 9.49	53.85 ± 2.34	65.44 ± 5.37	***0.000***
Disease course (m) [median (IQR)]	34 (16.5–56)	34.5 (17.25–63.25)	48 (27–90.5)	18 (14.25–31.5)	***0.029***[c]
Diagnostic delay (m) [median (IQR)]	5 (1–8.5)	5 (1.25–8.75)	5 (2–13.5)	4 (1–6)	0.526
Positive fatigue test, *n* (%)	57/64 (89.1%)	31/35 (88.6%)	12/13 (92.3%)	14/16 (87.5%)	1*
Positive neostigmine test, *n* (%)	41/51 (80.4%)	19/27 (70.4%)	10/11 (90.9%)	12/13 (92.3%)	0.230*
Fluctuating weakness, *n* (%)	48 (69.6%)	27 (67.5%)	11 (84.6%)	10 (62.5%)	0.427*
RNS test positive, *n* (%)	45/63 (71.4%)	26/34 (76.5%)	11/13 (84.6%)	8/16 (50.0%)	0.090*
MGFA classification at onset					0.644*
I, *n* (%)	18 (26.1%)	11 (27.5%)	4 (30.8%)	3 (18.8%)	
II, *n* (%)	42 (60.9%)	23 (57.5%)	9 (69.2%)	10 (62.5%)	
III, *n* (%)	6 (8.7%)	5 (12.5%)	0	1 (6.3%)	
IV, *n* (%)	1 (1.4%)	0	0	1 (6.3%)	
V, *n* (%)	2 (2.9%)	1 (2.5%)	0	1 (6.3%)	
MGFA classification at maximal worsening					0.321*
II, *n* (%)	17 (24.6%)	10 (25.0%)	4 (30.8%)	3 (18.8%)	
III, *n* (%)	25 (36.2%)	15 (37.5%)	4 (30.8%)	6 (37.5%)	
IV, *n* (%)	5 (7.2%)	2 (5.0%)	3 (23.1%)	0	
V, *n* (%)	22 (31.9%)	13 (32.5%)	2 (15.4%)	7 (43.8%)	
Comorbid disease					
Hypertension, *n* (%)	8 (11.6%)	1 (2.5%)	2 (15.4%)	5 (31.3%)	***0.006***[b,*]
Diabetes mellitus, *n* (%)	6 (8.7%)	0	1 (7.7%)	5 (31.3%)	***0.001***[b,*]
Hyperlipidemia, *n* (%)	5 (7.2%)	0	2 (15.4%)	3 (18.8%)	***0.013***[a,b,*]
Hepatitis B, *n* (%)	5 (7.2%)	4 (10.0%)	1 (7.7%)	0	0.579*
Latent tuberculosis, *n* (%)	2 (2.9%)	1 (2.5%)	0	1 (6.3%)	0.668*
Tumor, *n* (%)	1 (1.4%)	0	0	1 (6.3%)	0.420*
Other autoimmune disease					
Thyroid abnormalities, *n* (%)	8 (11.6%)	2 (5.0%)	0	6 (37.5%)	***0.003***[b,*]
Urticaria, *n* (%)	3 (4.3%)	2 (5%)	1 (7.7%)	0	0.762*
Eczema, *n* (%)	1 (1.4%)	1 (7.7%)	0	0	0.188*
Positive ANA, *n* (%)	11/41 (26.8%)	4/24 (16.7%)	2/6 (33.3%)	5/11 (45.5%)	**0.175***

[a] *Early-onset vs. late-onset.*
[b] *Early-onset vs. very-late-onset.*
[c] *Late-onset vs. very-late-onset.*
**Using Fisher exact test. The bold and italic values mean significant differences.*
RNS, repetitive nerve stimulation; MGFA, Myasthenia Gravis Foundation of America; ANA, antinuclear-antibody.

the highest 6 months after onset and was decreased to 9/59 6 months later, perhaps due to the immunotherapy.

Table 3 showed details of the treatment and prognosis of the patients. All patients were followed up with the median follow-up period of 32 [(IQR) 13.5–56)] months. Sixty-four (94.1%) patients received steroids, 25 (39.1%) received at least one non-steroid immunosuppressant, 46 (66.7%) received rituximab and 39 (60.9%) reached MSE status. Among the patients who received rituximab, 44 patients were administered 600 mg rituximab every 6 months and two patients from Xi'an Gaoxin Hospital used a regimen of 4 weekly infusions of 100 mg followed by maintenance therapy depending on the emergence of $CD20^{+}$B-cells. Sixteen patients (23.5%) were refractory MuSK-MG and 13/16 patients received rituximab and 8/13 reached MSE. Thirteen patients (19.1%) attained MSE status using conventional treatments. Twenty-six patients (38.2%) did not reach MSE status until the use of rituximab. Among 41 patients who did not reach the status of MSE before administrating RTX with 600 mg regimen, although no significant difference (log-rank test: $p = 0.075$), a trend that more patients in early course of disease ($\leq$1 year) reached the status of MSE was observed (**Supplementary Figure 1**). One patient underwent thymectomy and the histopathologic diagnosis was thymic hyperplasia.

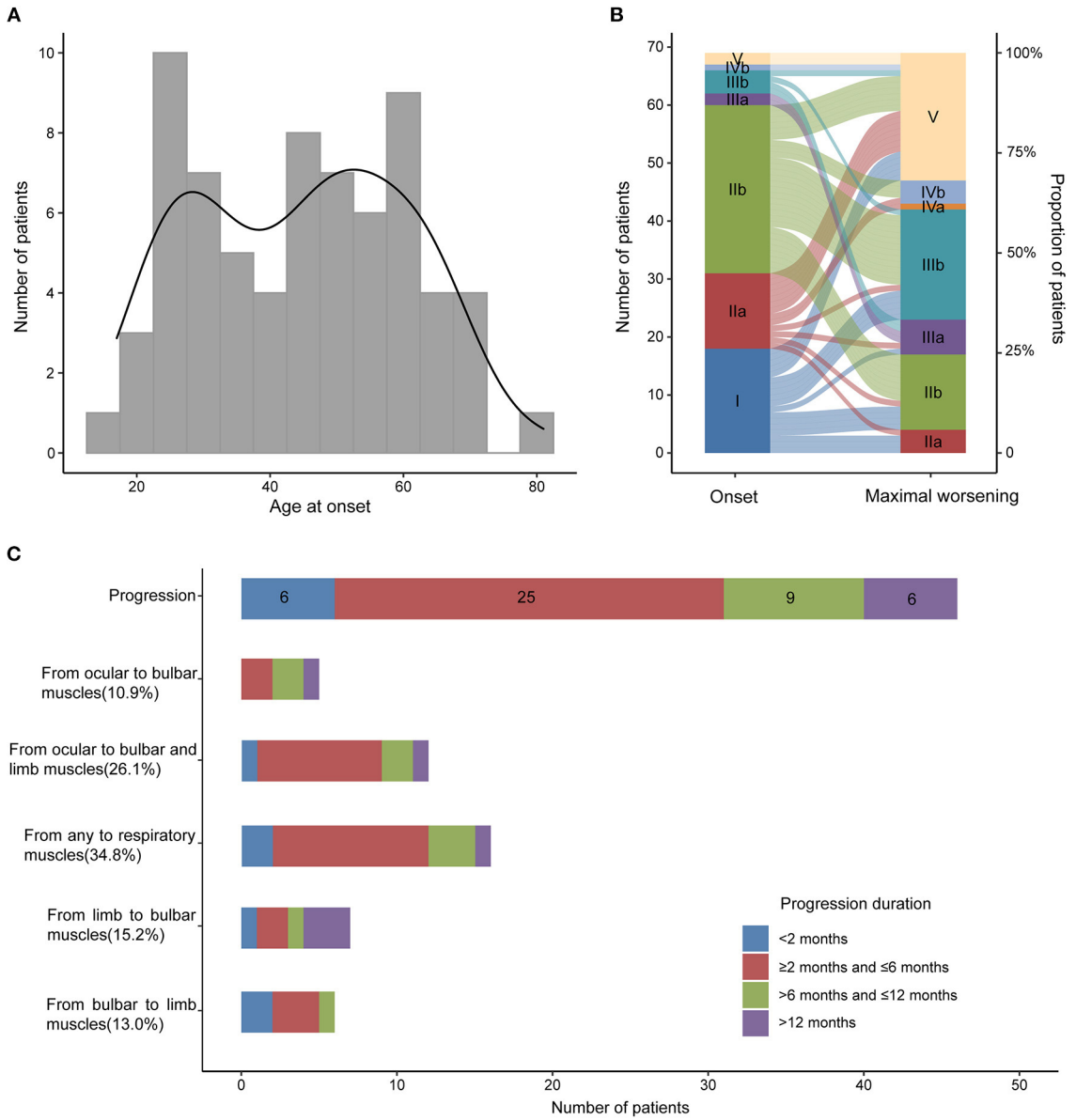


FIGURE 2 | Age at onset, MGFA classification at the onset and maximal worsening and disease progression in MuSK-MG. **(A)** Age at onset of 69 MuSK-MG patients in our cohort; **(B)** MGFA classification at the onset and at maximal worsening during the period from disease onset to the last follow-up; **(C)** Muscle involvement and disease progression of all the patients.

Difference Among Age Subgroups in MuSK-MG

According to age at onset, 40 patients (58%) were subclassified into early-onset, 13 (18.8%) into late-onset and 16 (23.2%) into very-late-onset subgroup. Clinical features of each subgroup were summarized in **Tables 1–3**. All subgroups were female-dominant and no difference of diagnostic delay was found. Among three subgroups, the positive rate of fatigue test and neostigmine test, the complaint of weakness fluctuating showed no difference, either. As for combined diseases, hypertension (5/16 vs. 1/40, $p = 0.006$), diabetes mellitus (5/16 vs. 0/40, $p = 0.001$) and hyperlipidemia (3/16 vs. 0/40, $p = 0.013$) occurred more frequently in very-late-onset subgroup than in early-onset subgroup. More patients in very-late-onset subgroup showed thyroid abnormalities (6/16 vs. 2/40, $p = 0.003$).

At disease onset, no differences were observed regarding MGFA classification and weakness distribution among three subgroups. Compared patients with early-onset, patients with very-late-onset onset showed a higher frequency of limb involvement (12/15 vs.16/40, respectively, $p = 0.022$) 3 months after onset. Six months after onset, more patients in

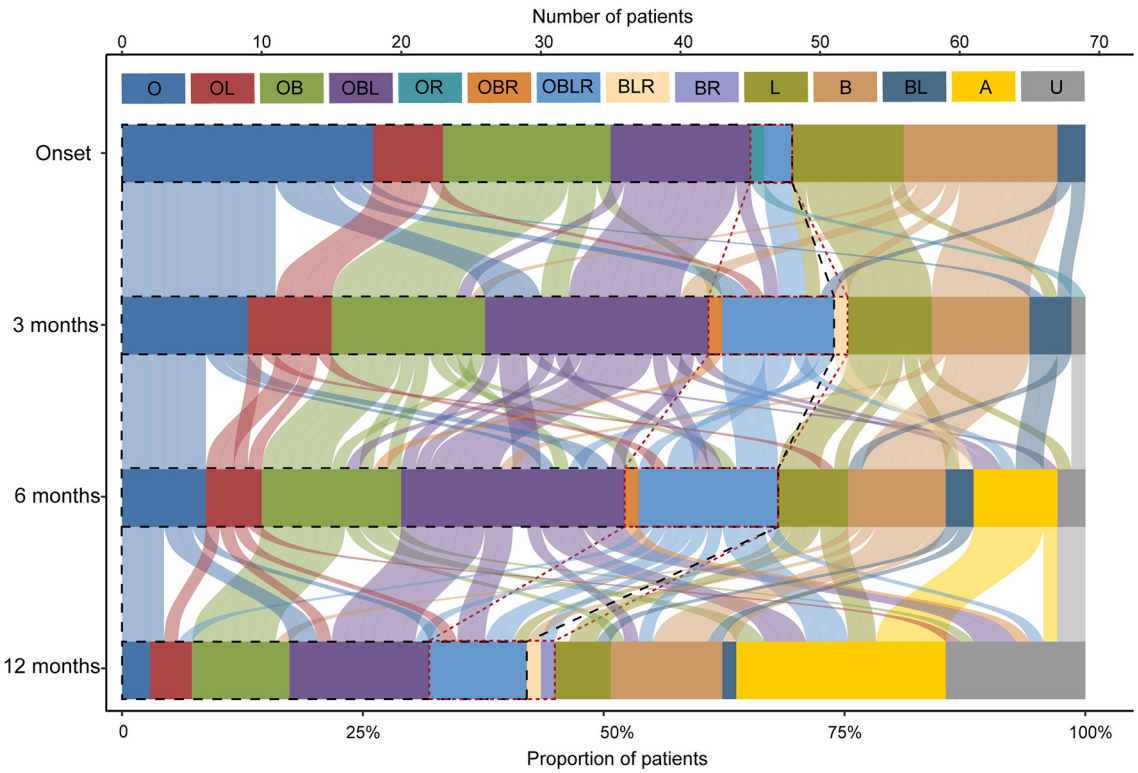


FIGURE 3 | Point-in-time weakness distribution and shifts over time. The point-in-time weakness distribution at disease onset and 3, 6, 12months after onset. O, extraocular muscles; B, bulbar muscles; L, limb or neck muscles; R, respiratory muscles; and their multiple combinations. A, asymptomatic; U, unknown, patients who were lost to follow-up at this time point. The black dotted line outlined the patients with ocular involvement and the red dotted line outlined the patients with respiratory involvement.

very-late-onset subgroup had bulbar and respiratory involvement than that in late-onset subgroup (bulbar: 14/15 vs. 26/39, $p = 0.044$; respiratory: 6/15 vs. 0/13, $p = 0.013$). In addition, more patients in very-late-onset subgroup showed weakness of limbs (86.7%, $p < 0.001$) than that in the other two subgroups. One year after onset, a higher frequency of respiratory involvement was reported in very-late-onset than in early-onset subgroups (4/12 vs. 2/35, $p = 0.036$) (**Table 2**). The time from onset to progression, the time from onset to maximal worsening, or from onset to MC among subgroups were not statistically different ($p > 0.05$).

Among age subgroups, the number of patients treated with ACEI, glucocorticoid, rituximab, PE, and IVIg was not significantly different. The proportion of refractory MuSK-MG did not differ from each other, either. The rate of patients who had reached MSE status (25/38, 9/13, 5/13, respectively, $p > 0.05$) and the time from onset to MSE status showed no significant difference (**Table 3**). For patients treated with rituximab, a shorter time from onset to receiving rituximab was found in very-late-onset subgroup compared to late-onset subgroup {6 [(IQR) 5.5–7.5] months vs. 18 [(IQR) 12–65] months, $p = 0.039$}. No significant difference in the rate to achieve MSE and the time from rituximab treatment to achieving MSE was identified among three subgroups ($p > 0.05$) (**Figure 4**).

DISCUSSION

As reported in previous studies, patients with MuSK-MG in our cohort also showed predominant involvement of extraocular, bulbar and respiratory muscles (7). However, the age at onset showed a bimodal age pattern of incidence, with one peak in individuals younger than 40 years, one peak in individuals aged 40–70 years old, which was different from the conclusion that the age at onset was rarely after 60 (7, 21, 22). The increase in the incidence of very-late-onset MuSK-MG might be a result of the aging of general population and expansion of life expectancy. The acknowledgment of clinical features of MuSK-MG and the increase in sensitivity and specificity of diagnostic methods also lead to an increase of diagnostic yield and a decline of misdiagnosis (19, 23, 24). It can also be attributable to the changes in the immune system during aging, including the increase in inflammatory reactions and the higher production of autoantibodies (25).

Growing evidence from clinical researches suggested the differences in clinical profile, natural history and treatment outcome among age subgroups in AChR-MG. Female cases outnumber male cases by three to one in early-onset patients (9). They were more likely to present with an initially generalized disease and a high level of anti-AChR antibodies associated

TABLE 2 | Weakness distribution and disease progression in early-onset, late-onset, and very-late-onset MuSK-myasthenia gravis.

Variables	Total *N* = 69	Early-onset *N* = 40	Late-onset *N* = 13	Very-late-onset *N* = 16	*P*-value
Weakness distribution at onset					
Extraocular, *n* (%)	48 (69.6%)	27 (67.5%)	10 (76.9%)	11 (68.8%)	0.877*
Bulbar, *n* (%)	37 (53.6%)	22 (55%)	7 (53.8%)	8 (50%)	0.945*
Limbs, *n* (%)	20 (29.0%)	9 (22.5%)	3 (23.1%)	8 (50%)	0.125*
Neck, *n* (%)	20 (29%)	11 (27.5%)	4 (30.8%)	5 (31.3%)	0.937*
Respiratory, *n* (%)	3 (4.3%)	1 (2.5%)	0	2 (12.5%)	0.221*
Weakness distribution 3 months after onset					
Extraocular, *n* (%)	51/68 (75%)	29 (72.5%)	10 (76.9%)	12/15 (80%)	0.926*
Bulbar, *n* (%)	47/68 (69.1%)	27 (67.5%)	8 (61.5%)	12/15 (80%)	0.543*
Limbs, *n* (%)	33/68 (48.5%)	16 (40%)	5 (38.5%)	12/15 (80%)	***0.022***[b,*]
Neck, *n* (%)	29/68 (42.6%)	18 (45%)	5 (38.5%)	6/15 (40%)	0.891*
Respiratory, *n* (%)	10/68 (14.7%)	5 (12.5%)	0	5/15 (33.3%)	0.038*
Weakness distribution 6 months after onset					
Extraocular, *n* (%)	47/67 (70.1%)	27/39 (69.2%)	8 (61.5%)	12/15 (80%)	0.579*
Bulbar, *n* (%)	47/67 (70.1%)	26/39 (66.7%)	7 (53.8%)	14/15 (93.3%)	***0.044***[c,*]
Limbs, *n* (%)	28/67 (41.8%)	12/39 (30.8%)	3 (23.1%)	13/15 (86.7%)	***0.000***[b,c,*]
Neck, *n* (%)	22/67 (32.8%)	11/39 (28.2%)	3 (23.1%)	8/15 (53.3%)	0.178*
Respiratory, *n* (%)	11/67 (16.4%)	5/39 (12.8%)	0	6/15 (40%)	***0.013***[c,*]
Weakness distribution 1 year after onset					
Extraocular, *n* (%)	29/59 (49.2%)	18/35 (51.4%)	6/12 (50%)	5/12 (41.7%)	0.934*
Bulbar, *n* (%)	35/59 (59.3%)	21/35 (60%)	8/12 (66.7%)	6/12 (50%)	0.715*
Limbs, *n* (%)	19/59 (32.2%)	10/35 (28.6%)	3/12 (25%)	6/12 (50%)	0.375*
Neck, *n* (%)	17/59 (28.8%)	9/35 (25.7%)	3/12 (25%)	5/12 (41.7%)	0.623*
Respiratory, *n* (%)	9/59 (15.3%)	2/35 (5.7%)	3/12 (25%)	4/12 (33.3%)	***0.036***[b,*]
Time from onset to maximal worsening (m), [median (IQR)]	4 (1–11.5)	4 (1–14)	7 (3.5–13)	2.5 (0.475–6)	0.288
Progress, *n* (%)	46 (66.7%)	26 (65%)	8 (61.5%)	12 (75%)	0.778*
Progression ≤ 6 months from onset, *n* (%)	31 (44.9%)	18 (45%)	3 (23.1%)	10 (62.5%)	0.105*
Time from onset to progression (m), median (IQR)	4.5 (2–9.25)	4 (2.75–7.5)	9.5 (3.25–39.5)	3.5 (2–6)	0.097
Myasthenic crisis, *n* (%)	22 (31.9%)	13 (32.5%)	2 (15.4%)	7 (43.8%)	0.271*
Myasthenic crisis ≤ 6 months from onset, *n* (%)	11 (15.9%)	6 (15%)	0	5 (31.3%)	0.088*
Time from onset to crisis (m) [median (IQR)]	7 (2.75–13)	8 (3.5–16.5)	10.5 (9-)	3 (1–11)	0.417

[b] *Early-onset vs. very-late-onset.*

[c] *Late-onset vs. very-late-onset.*

**Using Fisher exact test. The bold and italic values mean significant differences.*

with thymic follicular hyperplasia (10, 26). Late and very-late-onset AChR-MG was more common in males and more frequently had seropositive acetylcholine receptor antibodies and ocular MG (10, 11, 14, 27). The therapeutic management of these two groups is more complex because of comorbidities (28). But Cortés-Vicente et al. found although very-late-onset patients had a higher frequency of life-threatening events, their long-term outcomes were good, with less requirement for immunosuppressive medications and a lower probability of being refractory (10).

However, the difference of clinical features, longitudinal disease progression and treatment outcomes among age subgroups in MuSK-MG is not clear. In our cohort, patients in very-late-onset subgroups showed a higher proportion of combined chronic diseases, including hypertriton, diabetes, thyroid abnormalities, etc. There was no difference of MGFA classification and weakness distribution at disease onset, but patients in the very-late-onset subgroup showed an early involvement of limb, bulbar and respiratory muscles in the disease course, especially in the first 6 months. As a result, very-late-onset patients started rituximab treatment earlier. Concerning the treatment outcome, three subgroups attained similar outcomes with no significant difference in the rate and time of remission.

We found that the distinction among age subgroups in MuSK-MG was not as great as that in AChR MG, which might be

TABLE 3 | Treatment and prognosis in early-onset, late-onset, and very-late-onset MuSK-myasthenia gravis (MuSK-MG).

Variables	Total *N* = 69	Early-onset *N* = 40	Late-onset *N* = 13	Very-late-onset *N* = 16	*P*-value
Treatment					
PE, *n/N* (%)	27/68 (39.7%)	16/39 (41%)	3/13 (23.1%)	8/16 (50%)	0.325*
IVIg, *n/N* (%)	30/68 (44.1%)	19/39 (48.7%)	5/13 (38.5%)	6/16 (37.5%)	0.709*
ACEI, *n/N* (%)	64/67 (95.5%)	37/38 (97.4%)	12/13 (92.3%)	15/16 (93.8%)	0.398*
Steroid, *n/N* (%)	64/68 (94.1%)	37/39 (94.9%)	12/13 (92.3%)	15/16 (93.8%)	1*
Rituximab, *n/N* (%)	46/68 (66.7%)	20/39 (75.0%)	7/13 (53.8%)	9/16 (56.3%)	0.202*
MMF, *n/N* (%)	5/68 (7.4%)	1/39 (2.6%)	2/13 (15.4%)	2/16 (12.5%)	0.121*
Tacrolimus, *n/N* (%)	12/68 (17.6%)	7/39 (17.9%)	3/13 (23.1%)	2/16 (12.5%)	0.829*
AZA, *n/N* (%)	12/68 (17.6%)	8/39 (20.5%)	2/13 (15.4%)	2/16 (12.5%)	0.908*
CTX, *n/N* (%)	1/68 (1.5%)	0	1/13 (7.7%)	0	0.191*
Cyclosporine, *n/N* (%)	2/68 (2.9%)	0	1/13 (7.7%)	1/16 (6.3%)	0.178*
Thymectomy, *n/N* (%)	1/68 (1.5%)	1/39 (2.6%)	0	0	1*
Refractory, *n/N* (%)	16/68 (23.5%)	10/39 (25.6%)	3/13 (23.1%)	3/16 (18.8%)	0.925*
Rituximab, *n/N* (%)	13/16 (81.3%)	9/10 (90%)	3/3 (100%)	1/3 (33.3%)	0.143*
Median follow-up period (m), median (IQR)	32 (13.5–56)	33.5 (15.5–63.25)	48 (27–90.5)	15 (9.75–20.75)	***0.007*[a,b]**
MSE, *n/N* (%)	39/64 (60.9%)	25/38 (65.8%)	9/13 (69.2%)	5/13 (38.5%)	0.218*
Time from onset to MSE (m), median (IQR)	11 (7.0–28.0)	10 (7.0–27.5)	15 (12.0–49.5)	10 (8.5–23.5)	0.254
Time from onset to receiving rituximab (m), median (IQR)	9 (6.0–24.75)	10 (5.75–49.5)	18 (12–65)	6 (5.5–7.5)	***0.039*[b]**

[a] *Early-onset vs. very-late-onset.*

[b] *Late-onset vs. very-late-onset.*

**Using Fisher exact test. The bold and italic values mean significant differences.*

PE, plasma exchange; IVIg, intravenous immunoglobulin; ACEI, acetylcholinesterase inhibitors; MMF, mycophenolate mofetil; AZA, azathioprine; CTX, cyclophosphamide; MSE, minimal symptom expression.

due to distinct pathogenesis between AChR-MG and MuSK-MG: (1) There are functional and morphological abnormalities of the thymus in the pathogenesis of AChR-MG. B-cell infiltrations are associated with thymic hyperplasia of lymphoproliferative, which could be identified in more than 80% early-onset patients (26, 29). The thymus of late-onset patients usually shows normal-for-age atrophy. Although the mechanisms are not understood, the presence of anti-striational and anti-cytokine autoantibodies in late-onset patients strongly suggests similar role with thymoma (30, 31). In contrast, thymic hyperplasia and thymoma are rarely observed in MuSK-MG. (2) AChR antibodies are mainly of IgG1 and IgG3 subtypes, which can bind to C1q to activate the complement cascade. The number of anti-AChR antibody producer, including plasma cells and memory B cells, decreased in the elderly (32, 33). By comparison, MuSK-MG autoantibodies are mainly of the IgG4 subclass, which undergo Fab-arm exchange as a prerequisite for pathogenic capacity (34). They are produced by plasmablasts, which are found in similar proportions in all age subgroups (35). (3) Anti-AChR antibodies modulate myogenic markers and lead to impaired muscle regeneration, while the effect of anti-MuSK antibodies on regeneration remains unclear (36). It is noteworthy that satellite cells are quantitatively and functionally age-dependent, with a marked decline with age (37, 38), this might explain the rapid progression in patients with very-late-onset MuSK-MG.

This study has several limitations. First, this was a retrospective study, and therefore, potential selection bias, missing data bias and recall misclassification could not be avoided. Second, only MSE was used to evaluate the prognosis of MG, other prognostic outcomes such as the reduction in daily dosage of prednisone and the maintenance of asymptomatic were not analyzed. Third, the sample size of the cohort, especially in late-onset and very-late-onset subgroups, is still small. To better understand the distinction of clinical features, longitudinal disease progression and treatment outcome in MuSK-MG among age subgroups, further prospective studies with larger sample size are required.

In conclusion, our results are consistent with previous studies, which showed MuSK-MG patients usually manifested as acute onset and predominant bulbar and respiratory involvement with female dominance. Compared with late-onset patients, very-late-onset patients displayed an early involvement of limb, bulbar and respiratory muscles in the disease course, which might prompt their earlier usage of rituximab. The majority MuSK-MG patients can benefit from rituximab treatment regardless of age at onset.

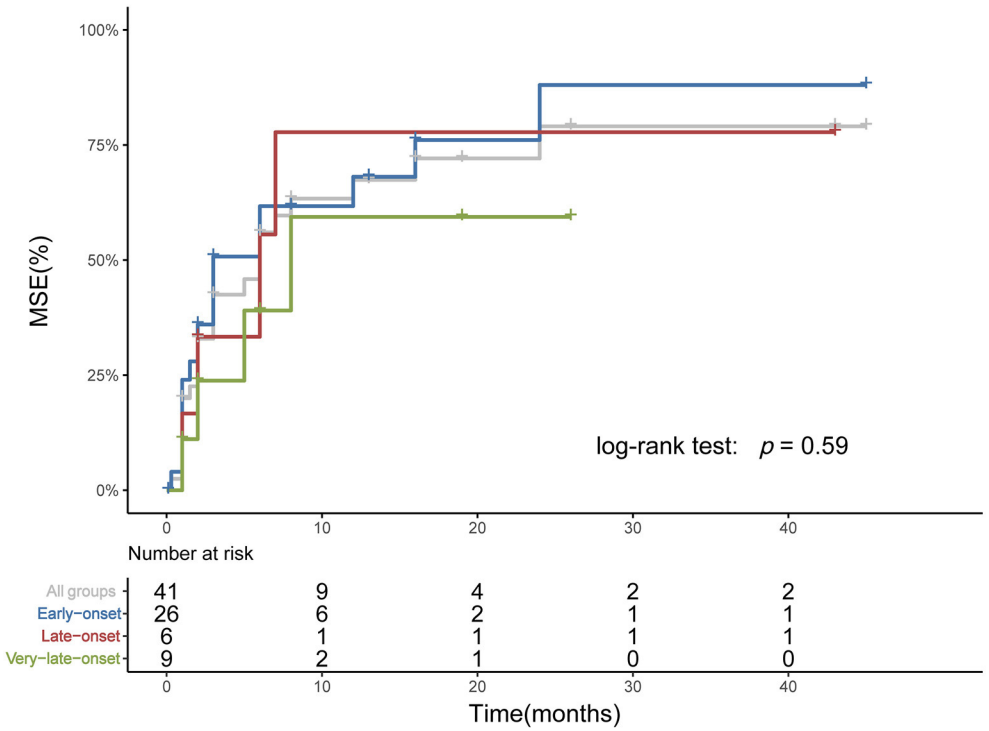


FIGURE 4 | Time from rituximab treatment to achieving MSE among subgroups. The Kaplan-Meier plot showed the time from rituximab treatment to reaching MSE. Three patients who had achieved MSE before rituximab and two patients from Xian Gaoxin Hospital with distinct regimen were not included. No significant difference was identified among subgroups.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Huashan Hospital, Fudan University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JX and WZ designed the study and revised the manuscript. CZ supervised the study. YZ and JC collected all the data and drafted the manuscript. CY conducted statistical analysis. ZL, ST, LZ, SL, JS, XH, and YW collected clinical and laboratory data. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by the National Natural Science Foundation of China (No. 81901279).

ACKNOWLEDGMENTS

The authors thank all the participants and their family members.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.879261/full#supplementary-material

REFERENCES

1. Oh SJ. Muscle-specific receptor tyrosine kinase antibody positive myasthenia gravis current status. *J Clin Neurol.* (2009) 5:53–64. doi: 10.3988/jcn.2009.5.2.53
2. Wolfe GI, Oh SJ. Clinical phenotype of muscle-specific tyrosine kinase-antibody-positive myasthenia gravis. *Ann N Y Acad Sci.* (2008) 1132:71–5. doi: 10.1196/annals.1405.005
3. Vincent A, Leite MI. Neuromuscular junction autoimmune disease: muscle specific kinase antibodies and treatments for myasthenia gravis. *Curr Opin Neurol.* (2005) 18:519–25. doi: 10.1097/01.wco.0000180660.57801.3f
4. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med.* (2001) 7:365–8. doi: 10.1038/85520

5. Evoli A, Alboini PE, Bisonni A, Mastrorosa A, Bartoccioni E. Management challenges in muscle-specific tyrosine kinase myasthenia gravis. *Ann N Y Acad Sci.* (2012) 1274:86–91. doi: 10.1111/j.1749-6632.2012.06781.x
6. Suh J, Goldstein JM, Nowak RJ. Clinical characteristics of refractory myasthenia gravis patients. *Yale J Biol Med.* (2013) 86:255–60. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3670444/
7. Hurst RL, Gooch CL. Muscle-specific receptor tyrosine kinase (MuSK) myasthenia gravis. *Curr Neurol Neurosci Rep.* (2016) 16:61. doi: 10.1007/s11910-016-0668-z
8. Nakata R, Motomura M, Masuda T, Shiraishi H, Tokuda M, Fukuda T, et al. Thymus histology and concomitant autoimmune diseases in Japanese patients with muscle-specific receptor tyrosine kinase-antibody-positive myasthenia gravis. *Eur J Neurol.* (2013) 20:1272–6. doi: 10.1111/ene.12169
9. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol.* (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3
10. Cortés-Vicente E, Álvarez-Velasco R, Segovia S, Paradas C, Casasnovas C, Guerrero-Sola A, et al. Clinical and therapeutic features of myasthenia gravis in adults based on age at onset. *Neurology.* (2020) 94:e1171–80. doi: 10.1212/WNL.0000000000008903
11. Li Y, Dong X, Li Z, Peng Y, Jin W, Zhou R, et al. Characteristics of myasthenia gravis in elderly patients: a retrospective study. *Neurol Sci.* (2021) 43:2775–2783. doi: 10.1007/s10072-021-05599-z
12. Evoli A, Batocchi AP, Minisci C, Di Schino C, Tonali P. Clinical characteristics and prognosis of myasthenia gravis in older people. *J Am Geriatr Soc.* (2000) 48:1442–8. doi: 10.1111/j.1532-5415.2000.tb02635.x
13. Wang W, Chen YP, Wei DN. [The clinical characteristics of early-onset versus late-onset types of myasthenia gravis]. *Zhonghua nei ke za zhi.* (2011) 50:496–8. doi: 10.3760/cma.j.issn.0578-1426.2011.06.013
14. Zivković SA, Clemens PR, Lacomis D. Characteristics of late-onset myasthenia gravis. *J Neurol.* (2012) 259:2167–71. doi: 10.1007/s00415-012-6478-6
15. Lavrnic D, Losen M, Vujic A, De Baets M, Hajdukovic LJ, Stojanovic V, et al. The features of myasthenia gravis with autoantibodies to MuSK. *J Neurol Neurosurg Psychiatry.* (2005) 76:1099–102. doi: 10.1136/jnnp.2004.052415
16. Barnett C, Bril V. New insights into very-late-onset myasthenia gravis. *Nat Rev Neurol.* (2020) 16:299–300. doi: 10.1038/s41582-020-0345-3
17. Jaretzki A III, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. *Ann Thorac Surg.* (2000) 70:327–34. doi: 10.1016/s0003-4975(00)01595-2
18. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. *Neurology.* (2016) 87:419–25. doi: 10.1212/WNL.0000000000002790
19. Kleiner-Fisman G, Kott HS. Myasthenia gravis mimicking stroke in elderly patients. *Mayo Clin Proc.* (1998) 73:1077–8. doi: 10.4065/73.11.1077
20. Wolfe GI, Herbelin L, Nations SP, Foster B, Bryan WW, Barohn RJ. Myasthenia gravis activities of daily living profile. *Neurology.* (1999) 52:1487–9. doi: 10.1212/WNL.52.7.1487
21. Sanders DB, El-Salem K, Massey JM, McConville J, Vincent A. Clinical aspects of MuSK antibody positive seronegative MG. *Neurology.* (2003) 60:1978–80. doi: 10.1212/01.WNL.0000065882.63904.53
22. Zhao S, Zhang K, Ren K, Lu J, Ma C, Zhao C, et al. Clinical features, treatment and prognosis of MuSK antibody-associated myasthenia gravis in Northwest China: a single-centre retrospective cohort study. *BMC Neurol.* (2021) 21:428. doi: 10.1186/s12883-021-02439-7
23. Vincent A, Clover L, Buckley C, Grimley Evans J, Rothwell PM. Evidence of underdiagnosis of myasthenia gravis in older people. *J Neurol Neurosurg Psychiatry.* (2003) 74:1105–1108. doi: 10.1136/jnnp.74.8.1105
24. Aarli JA. Myasthenia gravis in the elderly: is it different? *Ann N Y Acad Sci.* (2008) 1132:238–43. doi: 10.1196/annals.1405.040
25. Watad A, Bragazzi NL, Adawi M, Amital H, Toubi E, Porat BS, et al. Autoimmunity in the elderly: insights from basic science and clinics - a mini-review. *Gerontology.* (2017) 63:515–23. doi: 10.1159/000478012
26. Cron MA, Maillard S, Villegas J, Truffault F, Sudres M, Dragin N, et al. Thymus involvement in early-onset myasthenia gravis. *Ann N Y Acad Sci.* (2018) 1412:137–45. doi: 10.1111/nyas.13519
27. Aarli JA. Late-onset myasthenia gravis: a changing scene. *Arch Neurol.* (1999) 56:25–7. doi: 10.1001/archneur.56.1.25
28. Donaldson DH, Ansher M, Horan S, Rutherford RB, Ringel SP. The relationship of age to outcome in myasthenia gravis. *Neurology.* (1990) 40:786–90. doi: 10.1212/WNL.40.5.786
29. Leite MI, Jones M, Ströbel P, Marx A, Gold R, Niks E, et al. Myasthenia gravis thymus: complement vulnerability of epithelial and myoid cells, complement attack on them, and correlations with autoantibody status. *Am J Pathol.* (2007) 171:893–905. doi: 10.2353/ajpath.2007.070240
30. Marx A, Yamada Y, Simon-Keller K, Schalke B, Willcox N, Ströbel P, et al. Thymus and autoimmunity. *Semin Immunopathol.* (2021) 43:45–64. doi: 10.1007/s00281-021-00842-3
31. Huijbers MG, Marx A, Plomp JJ, Le Panse R, Phillips WD. Advances in the understanding of disease mechanisms of autoimmune neuromuscular junction disorders. *Lancet Neurol.* (2022) 21:163–75. doi: 10.1016/S1474-4422(21)00357-4
32. Caraux A, Klein B, Paiva B, Bret C, Schmitz A, Fuhler GM, et al. Circulating human B and plasma cells. Age-associated changes in counts and detailed characterization of circulating normal CD138- and CD138+ plasma cells. *Haematologica.* (2010) 95:1016–20. doi: 10.3324/haematol.2009.018689
33. Perez-Andres M, Paiva B, Nieto WG, Caraux A, Schmitz A, Almeida J, et al. Human peripheral blood B-cell compartments: a crossroad in B-cell traffic. *Cytom Part B Clin Cytom.* (2010) 78(Suppl. 1):S47–60. doi: 10.1002/cyto.b.20547
34. Fichtner ML, Jiang R, Bourke A, Nowak RJ, O'Connor KC. Autoimmune pathology in myasthenia gravis disease subtypes is governed by divergent mechanisms of immunopathology. *Front Immunol.* (2020) 11:776. doi: 10.3389/fimmu.2020.00776
35. Morbach H, Eichhorn EM, Liese JG, Girschick HJ. Reference values for B cell subpopulations from infancy to adulthood. *Clin Exp Immunol.* (2010) 162:271–9. doi: 10.1111/j.1365-2249.2010.04206.x
36. Attia M, Maurer M, Robinet M, Le Grand F, Fadel E, Le Panse R, et al. Muscle satellite cells are functionally impaired in myasthenia gravis: consequences on muscle regeneration. *Acta Neuropathol.* (2017) 134:869–88. doi: 10.1007/s00401-017-1754-2
37. Bigot A, Duddy WJ, Ouandaogo ZG, Negroni E, Mariot V, Ghimbovschi S, et al. Age-associated methylation suppresses SPRY1, leading to a failure of re-quiescence and loss of the reserve stem cell pool in elderly muscle. *Cell Rep.* (2015) 13:1172–82. doi: 10.1016/j.celrep.2015.09.067
38. Verdijk LB, Snijders T, Drost M, Delhaas T, Kadi F, van Loon LJ. Satellite cells in human skeletal muscle; from birth to old age. *Age.* (2014) 36:545–7. doi: 10.1007/s11357-013-9583-2

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 25 April 2022
doi: 10.3389/fneur.2022.857402

Lack of Immunotherapy as the Only Predictor of Secondary Generalization in Very-Late-Onset Myasthenia Gravis With Pure Ocular Onset

Sijia Zhao[1†], Xu Yan[1,2†], Jiaqi Ding[1†], Kaixi Ren[1], Shuyu Sun[1], Jiarui Lu[1], Chao Zhang[1], Kai Zhang[3], Zhuyi Li[1] and Jun Guo[1*]*

[1] Department of Neurology, Tangdu Hospital, Air Force Medical University, Xi'an, China, [2] Department of Neurology, Suide County Hospital, Yulin, China, [3] Department of Intensive Care Unit, Xi'an, Hospital, Xi'an, China

During the past two decades, an increasing number of patients with very-late-onset myasthenia gravis (v-LOMG) with an onset age of 65 years or older have been identified. However, few studies explore the predictors of secondary generalization in patients with v-LOMG with pure ocular onset. In this retrospective cohort study, 69 patients with v-LOMG were divided into ocular MG (OMG) and generalized MG (GMG), and the clinical characteristics and outcomes were compared. Cox regression analysis was performed to explore the predictors of generalization. The average onset age of the study population was 73.1 ± 4.2 years and the median disease duration was 48.0 months (interquartile range, 32.5–64.5 months). Serum acetylcholine receptor (AChR) antibody was detected in up to 86% of patients and concomitant diseases in approximately half of the patients. Male predominance was seen in OMG group while female predominance in GMG group ($p = 0.043$). Patients with OMG showed a lower positive rate of repetitive nerve stimulation (RNS) than those with GMG ($p = 0.014$), and favorable outcomes were obtained in more patients with OMG than those with GMG ($p < 0.001$). Of the 51 patients with pure ocular onset, 25 (49.0%) underwent secondary generalization. A higher probability of generalization was found in patients with positive RNS results and without immunotherapy ($p = 0.018$ and <0.001). Upon Cox regression analysis, immunotherapy was negatively associated with secondary generalization [HR (hazard ratio) 0.077, 95%CI [0.024–0.247], $p < 0.001$]. Altogether, compared to the patients with very-late-onset GMG, the counterparts with OMG exhibit a significantly higher female predominance and a lower positive rate of RNS tests, especially on facial and accessory nerves. Lack of immunotherapy is the only predictor of secondary generalization in those with pure ocular onset.

Keywords: very-late-onset, myasthenia gravis, predictor, immunotherapy, generalization

OPEN ACCESS

Edited by:
Hai-Feng Li,
Capital Medical University, China

Reviewed by:
Deepak Menon,
University Health Network (UHN), Canada
Michelangelo Maestri,
University of Pisa, Italy

***Correspondence:**
Jun Guo
guojun_81@163.com
Kai Zhang
1539451744@qq.com

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *18 January 2022*
Accepted: *22 March 2022*
Published: *25 April 2022*

Citation:
Zhao S, Yan X, Ding J, Ren K, Sun S, Lu J, Zhang C, Zhang K, Li Z and Guo J (2022) Lack of Immunotherapy as the Only Predictor of Secondary Generalization in Very-Late-Onset Myasthenia Gravis With Pure Ocular Onset. Front. Neurol. 13:857402. doi: 10.3389/fneur.2022.857402

INTRODUCTION

Myasthenia gravis (MG) is an organ-specific autoimmune disease characterized by the presence of pathogenic antibodies mainly targeting acetylcholine receptors (AChRs) located at the neuromuscular junctions, leading to fluctuating and fatigable weakness of skeletal muscles. With the onset age of 50 years as the boundary, MG is categorized into early-onset MG (EOMG) and late-onset MG (LOMG) with different demographic and clinical profiles, indicating the requirement for classification of this disease (1–3). In recent decades, owing to the extensive application of diagnostic testing and gradual improvement in living conditions, an increasing number of patients with very-late-onset MG (v-LOMG) with an onset age of 65 years or older have been identified (4), and patients with elderly onset appear to exhibit unique demographic and clinical characteristics from EOMG and LOMG (3, 5–8). It is noted that older age is more likely to be accompanied by comorbidities, more fragility to medication side effects, and aging-related changes of immune system (9, 10), which may influence the clinical phenotype. Hence, it is of great significance to further outline the picture of the subgroup with v-LOMG.

Based on the muscles involved, MG can be divided into ocular MG (OMG) and generalized MG (GMG). To date, secondary generalization in patients with pure ocular onset has been identified as a well-known hallmark of MG. Once generalized symptoms develop, the patient's clinical status would become worse and might be associated with a poorer prognosis. Although studies have indicated the importance of considering factors including onset age, AChR antibody status, thymoma, and immunotherapy as predictors of secondary generalization in patients with MG of different ages (11–15), risk factors for generalization in the population with v-LOMG have not been established as far. Herein, we conducted a retrospective cohort study enrolling patients with v-LOMG from a tertiary hospital in Northwest China to outline the clinical picture of v-LOMG in the Han Chinese population and explore the predictors of secondary generalization in this unique subgroup.

METHODS

Patient Enrollment and Data Collection

All patients with MG with an onset age of 65 years or older at outpatient and inpatient units of the Department of Neurology, Tangdu Hospital, between January 2017 and July 2020 were recruited in this study. The patients with complete medical and follow-up records were eventually enrolled after the written informed consent was obtained. **Figure 1** showed the flowchart of patient enrollment and grouping. A definite MG diagnosis was made based on the clinical symptoms of fluctuating, fatigable skeletal muscles weakness, and the evidence of at least one of the following items: (1) unequivocal response to cholinesterase inhibitor; (2) positive response to repetitive nerve stimulation (RNS) with amplitude decrement of >10% in compound muscle action potential; or (3) seropositivity for AChR antibody measured by radioimmunoprecipitation assay. The last follow-up visit was performed in August 2021 to ensure the disease duration of all the enrolled patients was 2 years or longer. Patients with confined ocular involvement till the last follow-up were defined as pure OMG, whereas those with pure ocular onset but undergoing secondary generalization were defined as transformed MG (TMG), and GMG consisted of TMG and those with generalized onset. Demographic data including gender, onset age, disease duration (from onset to the last follow-up), initial symptoms, AChR antibody status, RNS test results, thymic abnormalities on CT scan, and concomitant diseases at the initial contact were collected and then compared between OMG and GMG groups. Immunotherapy regimens in the course of disease were collected and divided into 3 groups: steroids only, steroids plus other immunosuppressants (IS), and IS only. In this study, IS included azathioprine, tacrolimus, mycophenolate mofetil, and intravenous immunoglobulin (IVIg). Clinical outcome was evaluated at the last follow-up (August 2021) by Myasthenia Gravis Foundation of America Post-Intervention Status (MGFA-PIS) and the achievement of minimal manifestations (MM) or better [including complete stable remission (CSR) and pharmacologic remission (PR)] was defined as favorable outcomes. A status of improved (I) was categorized as an intermediate outcome. Unchanged (U), worse (W), and exacerbation (E) were classified as unfavorable outcomes. Died (D) of MG was defined as a poor outcome. This study was approved by the Ethics Committees of Tangdu Hospital, Air Force Medical University (approval number: TDLL-KY-202105-04). Written informed consent was waived in accordance with the institutional requirements because of the retrospective nature of this study. As an alternative, oral informed consent to participate in this study was obtained from all the patients.

Statistical Analysis

Categorical variables were presented as number with percentage and numerical variables as mean with standard deviation (SD) or median with interquartile range (IQR). Statistical analysis was performed by the SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). Intergroup differences of categorical variables were evaluated by χ^2 test and Fisher's exact test when necessary, and those of continuous variables were compared by Student's *t*-test or Mann–Whitney U test. The probability of secondary generalization was presented using the Kaplan–Meier method and analyzed with the log-rank test. Cox regression analysis was performed on variables of interest to identify the predictors of secondary generalization. Hazard ratio (HR) with 95% confidence intervals (CI) was calculated. A value of $p < 0.05$ was considered statistically significant in all analyses.

RESULTS

General Information

As shown in **Figure 1**, 69 of the initially identified 86 patients entered into the final analysis. In general, this population with v-LOMG exhibited unique features such as high AChR antibody seropositivity in up to 86% of patients and predominant concomitant diseases in approximately 50% of patients (**Table 1**).

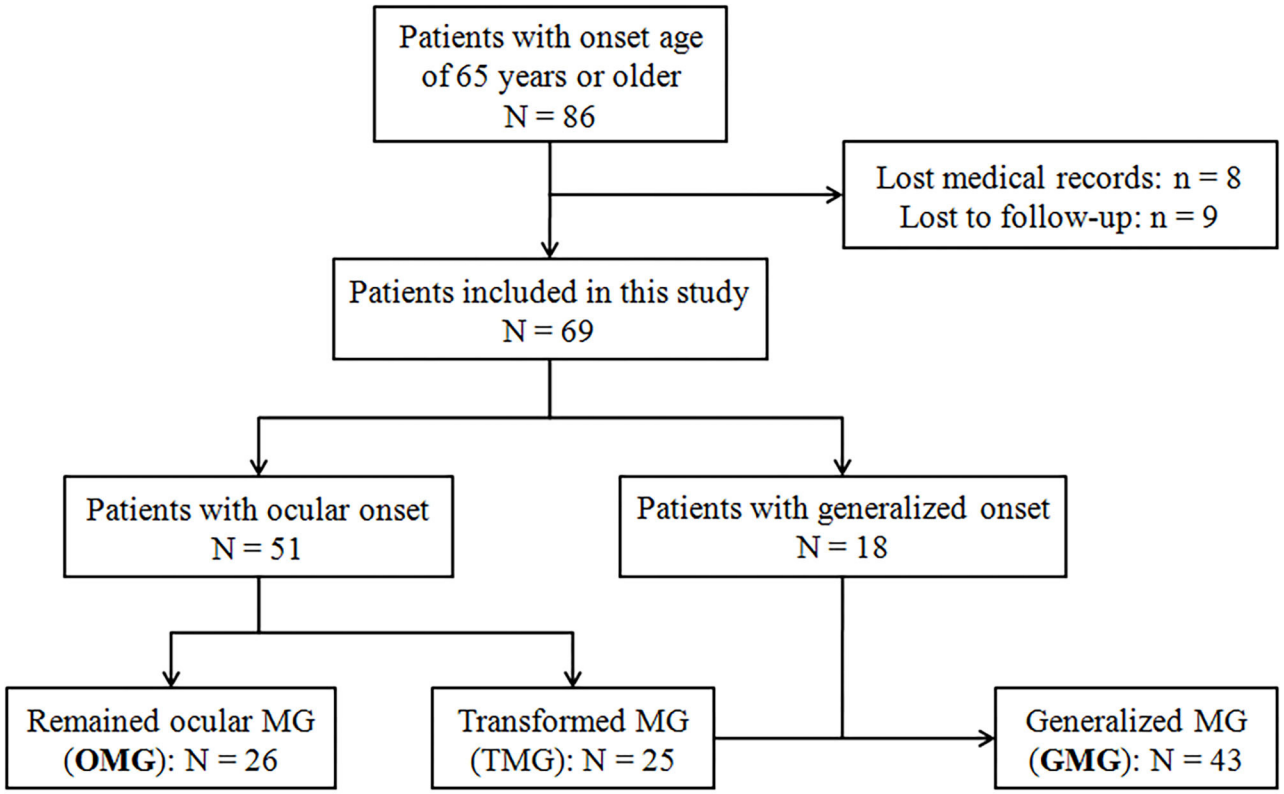


FIGURE 1 | Flowchart of the enrollment of the patient with v-LOMG in this study.

Comparison of Demographic and Clinical Characteristics Between Different v-LOMG Subtypes

In total, 69 patients with v-LOMG (32 females, 37 males) had an average onset age of 73.1 ± 4.2 years and a median disease duration of 48.0 months (IQR, 32.5–64.5 months). Although no obvious gender difference (female-to-male ratio, 1:1.2) was present in the entire study population, a male predominance was prominent in OMG group in contrast to that in GMG group ($p = 0.043$). Upon RNS tests, the positive result of any nerve was recorded in more patients with GMG than counterparts with OMG ($p = 0.014$). Specifically, the positive rates of RNS tests on facial and accessory nerves were significantly higher in GMG group than those in OMG group ($p = 0.013$ and 0.008, respectively) (**Table 1**). Considering the possibility of secondary generalization in patients with v-LOMG with pure ocular onset, we further compared the baseline demographic and clinical characteristics between the remained patients with OMG and those with TMG. As shown in **Table 2**, no significant intergroup differences were observed except for significantly higher positive rate of RNS tests in TMG group ($p = 0.032$, compared with OMG group), in particular when repetitive stimulating facial and accessory nerves ($p = 0.033$ and 0.020, respectively).

Comparison of Long-Term Outcomes Between Different v-LOMG Phenotypes or Therapies

First, we compared the long-term outcomes between OMG and GMG groups irrespective of the therapies used. Favorable outcomes were obtained in a significantly higher proportion of patients in OMG group than in GMG group (92.3% vs. 48.8%, $p < 0.001$; **Table 1**). Then the study population was divided into two groups based on whether or not receiving immunotherapy in the course of disease and the duration of therapy (short-term, <6 months, vs. long-term, ≥6 months), respectively, and clinical outcomes were compared between groups. Meanwhile, the outcomes associated with different treatment strategies were analyzed. Although no significant differences in the proportion of patients with distinct outcomes were observed between different duration of therapy (**Supplementary Table 1**) and amongst distinct treatment strategies (**Supplementary Table 2**), immunotherapy indeed led to a significantly higher proportion of favorable outcome and a lower proportion of unfavorable outcome compared with those not receiving immunotherapy (71.4% vs. 0, $p = 0.001$ and 4.8% vs. 50%, $p = 0.007$; **Supplementary Table 1**). Moreover, patients receiving immunotherapy showed a lower

TABLE 1 | Demographic, clinical characteristics, and long-term outcomes of the patients with v-LOMG.

Variables	MG	OMG	GMG	*P*-value
Gender	*N* = 69	*N* = 26	*N* = 43	
Male, *n* (%)	37 (53.6)	18 (69.2)	19 (44.2)	0.043
Female, *n* (%)	32 (46.4)	8 (30.8)	24 (55.8)	
Onset age (*y*), mean ± SD	73.1 ± 4.2	73.4 ± 3.9	72.9 ± 4.4	0.641
Disease duration (*m*), median (IQR)	48.0 (32.5–64.5)	49.5 (32.8–62.5)	45.0 (32.0–85.0)	0.985
Muscles initially involved	*N* = 69	*N* = 26	*N* = 43	
Ocular, *n* (%)	65 (94.2)	26 (100)	39 (90.7)	0.289*
Limbs, *n* (%)	7 (10.1)	NA	7 (16.3)	NA
Bulbar, *n* (%)	15 (21.7)	NA	15 (21.7)	NA
Axial muscles, *n* (%)	5 (7.2)	NA	5 (11.6)	NA
AChR antibody status	*N* = 63	*N* = 22	*N* = 41	
Positive, *n* (%)	54 (85.7)	20 (90.9)	34 (82.9)	0.476*
Negative, *n* (%)	9 (14.3)	2 (9.1)	7 (17.1)	
RNS test positive	*N* = 69	*N* = 26	*N* = 43	
Facial nerve, *n* (%)	29 (42.0)	6 (23.1)	23 (53.4)	0.013
Ulnar nerve, *n* (%)	6 (9.2)	1 (3.8)	5 (11.6)	0.398*
Axillary nerve, *n* (%)	30 (43.5)	8 (30.8)	22 (51.2)	0.098
Accessory nerve, *n* (%)	21 (30.4)	3 (11.5)	18 (41.9)	0.008
Any nerve, *n* (%)	42 (60.9)	11 (42.3)	31 (72.1)	0.014
Thymic abnormalities	*N* = 69	*N* = 26	*N* = 43	
Thymoma, *n* (%)	4 (5.8)	0 (0)	4 (9.3)	0.289*
Thymic hyperplasia, *n* (%)	18 (26.1)	8 (30.8)	10 (23.3)	0.491
Thymectomy, *n* (%)	6 (8.7)	0 (0)	6 (8.7)	0.067*
Concomitant diseases	*N* = 69	*N* = 26	*N* = 43	
Hypertension, *n* (%)	35 (50.7)	12 (46.2)	23 (53.5)	0.555
Diabetes, *n* (%)	20 (29.0)	8 (30.8)	12 (27.9)	0.800
Coronary heart disease, *n* (%)	13 (18.8)	6 (23.1)	7 (16.3)	0.535*
Cerebral infarction, *n* (%)	12 (17.4)	3 (11.5)	9 (20.0)	0.514*
Tumor, *n* (%)	4 (5.8)	1 (3.8)	3 (7.0)	1.000*
Immunotherapy	*N* = 63	*N* = 24	*N* = 39	
Steroids, *n* (%)	9 (13.0)	3 (11.5)	6 (14.0)	1.000*
Steroids + IS, *n* (%)	46 (66.7)	20 (76.9)	26 (60.5)	0.148
IS, *n* (%)	8 (11.6)	1 (3.8)	7 (16.3)	0.141*
Outcomes	*N* = 69	*N* = 26	*N* = 43	
Favorable, *n* (%)	45 (65.2)	24 (92.3)	21 (48.8)	< 0.001
Intermediate, *n* (%)	12 (17.4)	0 (0)	12 (27.9)	0.002*
Unfavorable, *n* (%)	6 (8.7)	2 (7.7)	4 (9.3)	1.000*
Poor, *n* (%)	6 (8.7)	0 (0)	6 (14.0)	0.076*
Myasthenic crisis, *n* (%)	5 (7.2)	0 (0)	5 (11.6)	0.149*

*AChR, Acetylcholine receptor; GMG, generalized myasthenia gravis; IQR, interquartile range; MG, myasthenia gravis; m, month; NA, not applicable; OMG, ocular myasthenia gravis; RNS, repetitive nerve stimulation; IS, immunosuppressant; SD, standard deviation; v-LOMG, very-late-onset myasthenia gravis; y, year. Outcomes were evaluated by the Myasthenia Gravis Foundation of America Post-Intervention Status (MGFA-PIS). Favorable outcomes were defined as the achievement of minimal manifestations (MM) or better, including complete stable remission (CSR), pharmacologic remission (PR), and MM. An intermediate outcome was considered as a status of improved (I); unfavorable outcomes as unchanged (U), worse (W), and exacerbation (E); and a poor outcome as died (D) of MG. Intergroup difference of onset age was analyzed by Student's t-test and that of disease duration by Mann–Whitney U test. Otherwise, χ^2 test was used to compare the intergroup differences. *Fisher's exact test was performed. The values of p were drawn from the statistical analysis between the OMG and GMG groups.*

proportion of developing myasthenic crisis than those not receiving immunotherapy (1.4% vs. 5.8%, $p < 0.001$).

Secondary Generalization of v-LOMG With Pure Ocular Onset

Of 69 patients, 51 (73.9%) initiated with pure ocular involvement, and the demographic and clinical characteristics of these patients are shown in **Table 2**. Of the 51 patients with pure ocular onset, 25 (49.0%) underwent secondary generalization. The cumulative survival without generalization was assessed by the Kaplan–Meier method (**Figure 2A**). Of note, secondary generalization occurred in nearly half of all 25 patients (48.0%) during the first 6 months after onset, 18 (72.0%) patients within 2 years, and 23 (92.0%) within 4 years (**Figure 2B**).

TABLE 2 | Baseline demographic and clinical data of patients with v-LOMG with pure ocular onset.

Variables	Ocular-onset MG	OMG	TMG	*P*-value
Gender	*N* = 51	*N* = 26	*N* = 25	
Male, *n* (%)	31 (53.6)	18 (69.2)	13 (52.0)	0.208
Female, *n* (%)	32 (46.4)	8 (30.8)	12 (48.0)	
Onset age (y), mean ± SD	72.7 ± 4.3	73.4 ± 3.9	72.0 ± 4.5	0.223
Disease duration (m), median (IQR)	49.0 (33.0–66.0)	49.5 (32.8–62.5)	48.0 (44.5–94.5)	0.423
Initial clinical symptoms	*N* = 51	*N* = 26	*N* = 25	
Unilateral ptosis, *n* (%)	29 (56.9)	16 (61.5)	13 (52.0)	0.492
Bilateral ptosis, *n* (%)	2 (3.9)	0 (0)	2 (8.0)	0.235*
Unilateral ptosis with diplopia, *n* (%)	16 (31.4)	7 (26.9)	9 (36.0)	0.485
Bilateral ptosis with diplopia, *n* (%)	4 (7.8)	3 (11.5)	1 (4.0)	0.610*
AChR antibody status	*N* = 45	*N* = 22	*N* = 23	
Positive, *n* (%)	39 (86.7)	20 (90.9)	19 (82.6)	0.665*
Negative, *n* (%)	6 (13.3)	2 (9.1)	4 (17.4)	
RNS test positive	*N* = 51	*N* = 26	*N* = 25	
Facial nerve, *n* (%)	19 (37.3)	6 (23.1)	13 (52.0)	0.033
Ulnar nerve, *n* (%)	3 (5.9)	1 (3.8)	2 (8.0)	0.610*
Axillary nerve, *n* (%)	21 (41.2)	8 (30.8)	13 (52.0)	0.124
Accessory nerve, *n* (%)	13 (25.5)	3 (11.5)	10 (40.0)	0.020
Any nerve, *n* (%)	29 (56.9)	11 (42.3)	18 (72.0)	0.032
Thymic abnormalities	*N* = 51	*N* = 26	*N* = 25	
Thymoma, *n* (%)	2 (3.9)	0 (0)	2 (8.0)	0.235*
Thymic hyperplasia, *n* (%)	12 (26.1)	8 (30.8)	4 (16.0)	0.214
Thymectomy, *n* (%)	3 (5.9)	0 (0)	3 (12.0)	0.110
Concomitant diseases	*N* = 51	*N* = 26	*N* = 25	
Hypertension, *n* (%)	29 (56.9)	12 (46.2)	17 (68.0)	0.115
Diabetes, *n* (%)	15 (29.4)	8 (30.8)	7 (28.0)	0.828
Coronary heart disease, *n* (%)	10 (19.6)	6 (23.1)	4 (16.0)	0.726*
Cerebral infarction, *n* (%)	10 (19.6)	3 (11.5)	7 (28.0)	0.173*
Tumor, *n* (%)	4 (7.8)	1 (3.8)	3 (12.0)	0.350*
Immunotherapy	*N* = 47	*N* = 24	*N* = 23	
Time from onset to immunotherapy initiation (m), median (IQR)	5.0 (1.0–24.0)	4.0 (1.0–17.3)	7.0 (2.5–24.0)	0.156

*AChR, Acetylcholine receptor; IQR, interquartile range; m, month; OMG, ocular myasthenia gravis; RNS, repetitive nerve stimulation; SD, standard deviation; TMG, transformed myasthenia gravis; v-LOMG, very-late-onset myasthenia gravis; y, year. Intergroup difference of onset age was analyzed by Student's t-test and those of disease duration and time from onset to immunotherapy initiation by Mann–Whitney U test. Otherwise, χ^2 test was used to compare the intergroup differences. *Fisher's exact test was performed. The values of p were drawn from statistical analysis between the OMG and TMG groups.*

Probability of Secondary Generalization of v-LOMG With Pure Ocular Onset

There were no significant differences in the cumulative probabilities of generalization between male and female patients ($p = 0.131$; **Figure 3A**), those with positive and negative AChR antibody ($p = 0.792$; **Figure 3B**), those with and without thymic abnormalities ($p = 0.206$; **Figure 3C**), and those with and without concomitant diseases ($p = 0.169$; **Figure 3E**), respectively. In contrast, significantly higher probabilities were found in patients with positive RNS results than those with negative results ($p = 0.018$) (**Figure 3D**). Fifty-one patients with ocular-onset were divided into two groups based on whether or not receiving immunotherapy before generalization and entered into statistical analysis. As revealed in **Figure 3F**, patients not receiving immunotherapy had a significantly higher probability of generalization than those receiving immunotherapy ($p < 0.001$).

We further assessed the intervals from pure ocular onset to generalization in the 25 patients undergoing secondary generalization. Patients with positive AChR antibody, positive RNS results, and not receiving immunotherapy had a shorter time to generalization than those with negative AChR antibody, negative RNS results, and receiving immunotherapy ($p = 0.016$, 0.007, and 0.010, respectively; **Figures 4B,C,F**), whereas no significant differences were observed between male and female patients ($p = 0.766$; **Figure 4A**), those with and without concomitant diseases ($p = 0.916$; **Figure 4D**), and those with and without thymic abnormalities ($p = 0.113$; **Figure 4E**).

Predictors of Secondary Generalization in Patients With v-LOMG

Upon Cox regression analysis, acetylcholinesterase inhibitor (AChEI) was excluded because it had been given to all patients. As

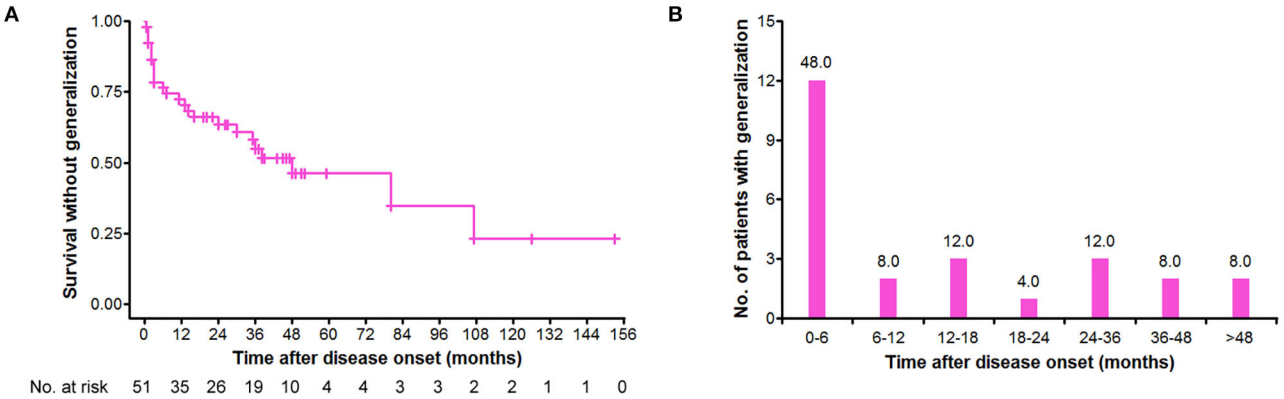


FIGURE 2 | Distribution of secondary generalization in the patients with v-LOMG with pure ocular onset. **(A)** Kaplan–Meier curve depicting the cumulative survival without generalization over time (months). **(B)** Time distribution of generalization in the 25 patients with v-LOMG. *Y*-axis indicates the number of patients undergoing generalization with the percentage showing on the top of each column.

revealed in **Table 3**, a total of 10 variables of interest were selected to explore the potential risk factors for secondary generalization. Among these, immunotherapy was the only predictor negatively associated with secondary generalization in patients with v-LOMG with pure ocular onset (HR 0.077, 95%CI [0.024–0.247], $p < 0.001$).

DISCUSSION

In recent years, an accumulating body of studies has demonstrated a true biologic increase in the incidence of elderly onset MG and primarily ascribes this increase to dramatically increased longevity, the aging immune system, and improved diagnostic measures (16–19). Till now, there is a lack of consensus on the definition of this subgroup, and in most studies, the cutoff onset age was defined at 65 or 70 years (7, 19–21). In this study, we included patients with MG with an onset age of 65 years or older and outlined the picture of this subgroup with v-LOMG.

Male predominance is widely recognized in population with elderly onset MG from the Western countries (4, 20–22), whereas female predominance in a Japanese nationwide survey (19), possibly owing to the differences in racial and genetic backgrounds. In this study, a mild male predominance in a small sample of Chinese patients, together with distinct male-to-female ratios in OMG (2.3:1) and GMG (1:1.3) groups further indicate potential gender predominance dependent on clinical subtypes (23). Previous studies enrolling elderly onset MG showed a high prevalence of AChR antibodies ranging from 80% to nearly 93% (4, 6, 19, 21). Similarly, our study showed a comparable positive rate of 85.7%. However, inconsistent with the reported positive rate of 30–77% in the entire OMG population (24), the higher rate of 90.9% in our ocular v-LOMG cohort suggests the role of aging-related changes in the strength of immune response on the differences. Of note, the higher prevalence of AChR antibody in patients with ocular v-LOMG might imply a tendency of secondary generalization in the future. Besides, a higher proportion of GMG than OMG was observed in our cohort. Although this finding is consistent with those from other patient cohorts (4, 19, 21), we cannot completely eliminate the possibility of underdiagnosis of OMG as a result of the ignorance of subtle ocular deficits at the early stage of disease by patients themselves and by clinicians (25).

It is generally accepted that older onset age, positive AChR antibody, the concurrence of thymoma, the use of AChEI, immunotherapy, and smoking can predict secondary generalization of patients with MG with pure ocular onset (3, 8, 26). However, it remains unclear whether such elements have the same effects in the v-LOMG subgroup, given the existence of age-related changes in immune intolerance (27). In this study, Cox regression analysis revealed that immunotherapy was the only predictor negatively associated with secondary generalization, reflecting the most crucial role of immunotherapy in improving the prognosis of v-LOMG. Of particular concern is that, as a specific parameter of elderly patients, concomitant diseases were illustrated not to be associated with a higher probability of secondary generalization. This reflects that no difference in the rate of concomitant diseases was present between patients with pure ocular onset undergoing and not undergoing secondary generalization.

Conclusions drawn from our study and other retrospective studies (9, 18, 21) supported the benefit of immunotherapy in treating patients with v-LOMG; however, in the process of achieving a good prognosis, clinicians often face decision-making difficulties and potential risks. Although our study revealed concomitant diseases were not associated with secondary generalization in patients with v-LOMG, this remains a major consideration in choosing the appropriate treatment strategy. Clinicians tend to be reluctant to treat patients with v-LOMG with an aggressive therapeutic protocol. For elderly patients receiving various medications for comorbidities, added-on immunotherapy might bring undesirable pharmacokinetic and pharmacodynamic drug interactions (28). The fragile and declining immunocompetence in the elderly potentially worsens

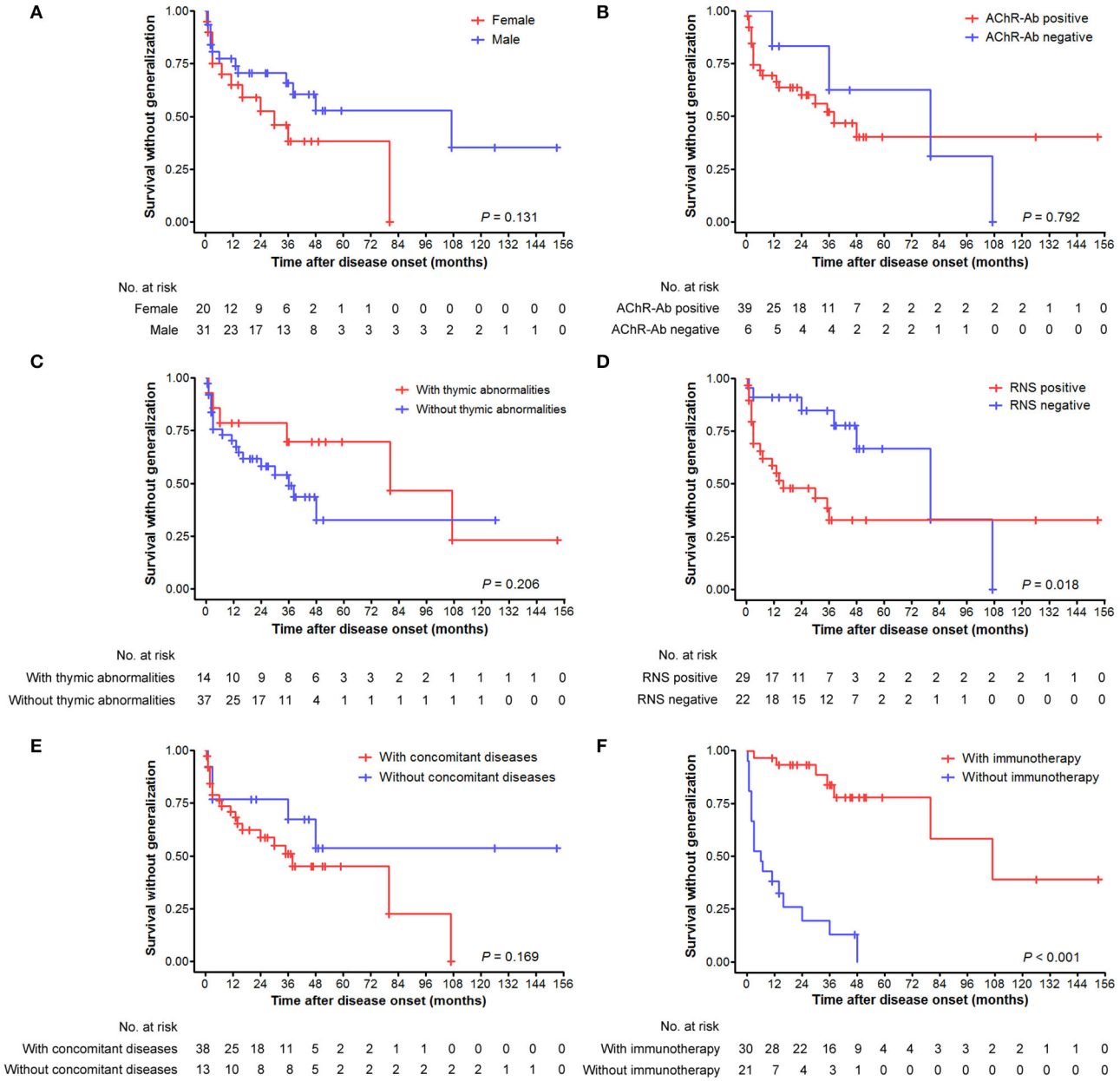


FIGURE 3 | Cumulative survivals without generalization over time (months) as depicted by the Kaplan–Meier curve. **(A)** Comparison between male and female patients. **(B)** Comparison between patients with positive and negative AChR antibody. **(C)** Comparison between patients with and without thymic abnormalities. **(D)** Comparison between patients with positive and negative RNS test results. **(E)** Comparison between patients with and without concomitant diseases. **(F)** Comparison between patients with and without immunotherapy before generalization.

the situation with the rise of drug-related complications and even leads to increased mortality risk (24, 29, 30). Despite these concerns, the satisfactory efficacy of immunotherapy in v-LOMG subgroup has been reported by several studies (31–33), and the treatment strategy of combining AChEI and rapid immunosuppression followed by chronic immunosuppression was recommended (9). This view was supported by our study where 71.4% of patients treated with AChEI plus immunotherapy achieved favorable outcomes. Furthermore, of 5 patients undergoing myasthenic crisis, only one had been treated with ISs accounting for 1.6% of all patients receiving immunotherapy, in contrast to other 4 patients not receiving immunotherapy (66.7%, $p < 0.001$), indicating the essential role of immunotherapy in preventing serious adverse consequences.

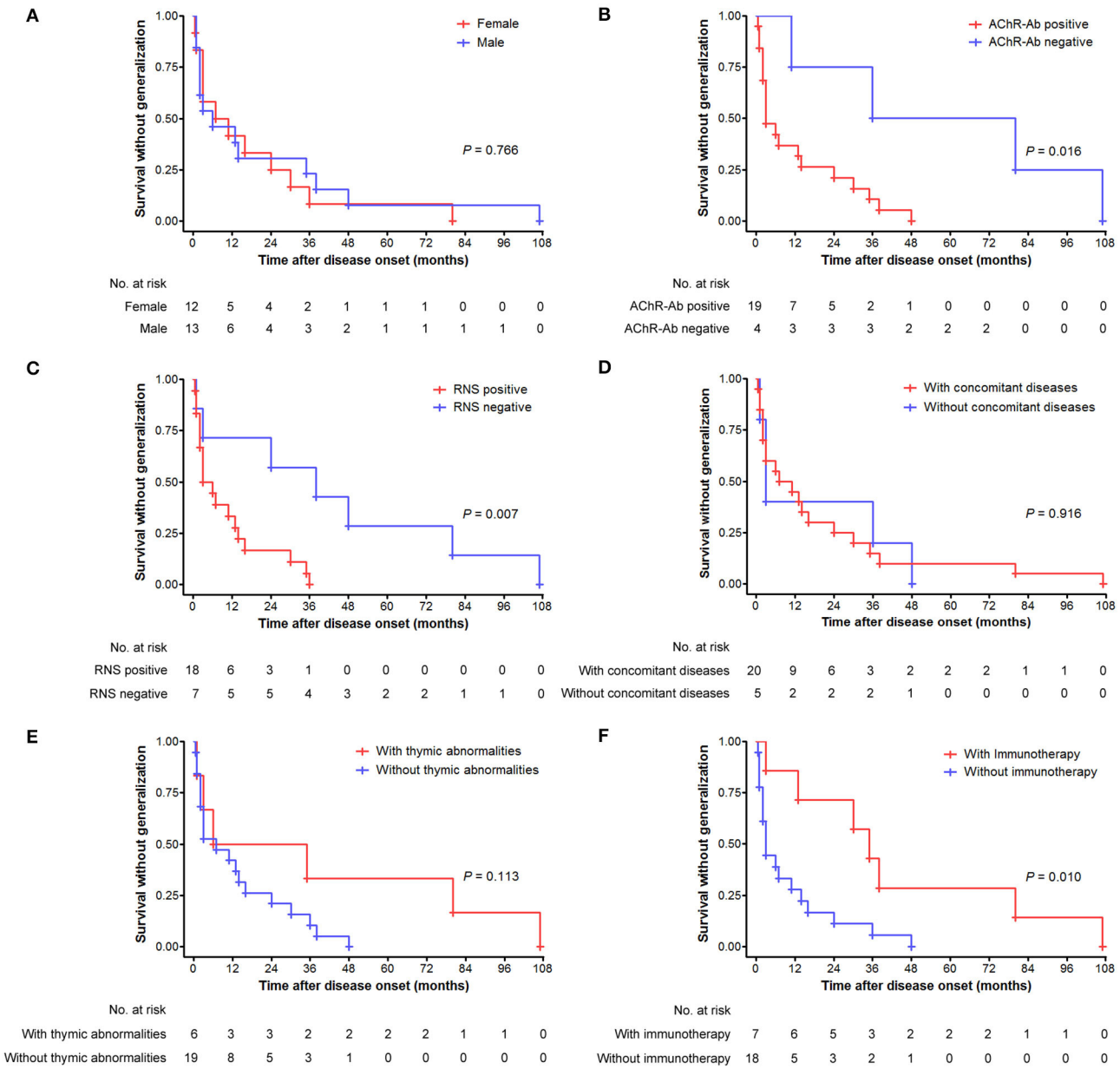


FIGURE 4 | Generalization tempo from disease onset over time (months) as depicted by the Kaplan–Meier curve. **(A)** Comparison between male and female patients. **(B)** Comparison between patients with positive and negative AChR antibody. **(C)** Comparison between patients with positive and negative RNS test results. **(D)** Comparison between patients with and without concomitant diseases. **(E)** Comparison between patients with and without thymic abnormalities. **(F)** Comparison between patients with and without immunotherapy before generalization.

Interestingly, there were no significant differences in the percentage of favorable outcomes amongst the steroids alone, immunosuppressant alone, and combined treatment groups. The predictive value of time from onset to immunotherapy initiation was not demonstrated on Cox regression analysis. These findings might be attributed to the small size of our patient cohort, the diversity of immunotherapy selection, and the feasibility of individualized treatment based on the benefit–risk assessment in a real-world setting. Even though the limitations are present, our observation that 80% of myasthenic crises had occurred before the initiation of immunotherapy still highlights the importance of immunosuppression as early as possible. Meanwhile, serious complications associated with immunotherapy cannot be ignored in the elderly, and prophylaxis against side effects of medications should be used to minimize the potential risks.

TABLE 3 | Cox regression analysis of risk factors for secondary generalization in patients with v-LOMG with pure ocular onset.

Variables	HR	95%CI	*P*-value
Gender, male vs. female	0.691	0.243–1.962	0.487
Onset age	0.944	0.842–1.058	0.319
Ptosis at onset, unilateral vs. bilateral	1.086	0.235–5.016	0.916
Diplopia at onset, yes vs. no	1.045	0.362–3.017	0.935
AChR antibody, positive vs. negative	1.254	0.226–6.950	0.795
RNS test, positive vs. negative	2.188	0.756–6.332	0.149
Thymic abnormalities, with vs. without	0.778	0.223–2.723	0.695
Concomitant diseases, with vs. without	1.295	0.416–4.027	0.656
Immunotherapy before generalization, with vs. without	0.077	0.024–0.247	<0.001
Time from onset to immunotherapy initiation	0.987	0.971–1.003	0.122

AChR, acetylcholine receptor; CI, confidential interval; v-LOMG, very-late-onset myasthenia gravis; HR, hazard ratio; RNS, repetitive nerve stimulation.

There are several limitations in this study. First, there was a lack of a unified schedule regarding the coverage of examinations and the timing of follow-up visits, given the nature of this retrospective cohort study. As a result, 8 patients were excluded due to incomplete medical records, and other 9 patients were lost to follow-up. The high exclusion rate of approximately 20% will inevitably affect the strength of our conclusion to some extent. Second, this study included 69 patients with v-LOMG from a single center and only 6 patients did not receive immunotherapy over the course of disease. Meanwhile, there was a lack of muscle-specific kinase antibody-associated MG (MuSK-MG) subgroup that may present distinct clinical features and responses to immunotherapy. The small number and single origin of patients may limit the significance of our conclusion and its scope of application. Third, Cox regression analysis revealed that positive RNS results were close to the borderline level of statistical significance. Considering the recognized predictive value of this variable on secondary generalization in prior studies (11, 26), our finding from v-LOMG subgroup requires further confirmation. Therefore, multicenter, prospective studies involving a larger sample of patients with v-LOMG originated from a wider geographical area are needed in future.

In conclusion, compared to patients with very-late-onset GMG, the counterparts with OMG exhibit a significantly higher female predominance and a lower positive rate of RNS tests, especially on facial and accessory nerves. Notably, lack of immunotherapy is the only predictor of secondary generalization in those patients with v-LOMG with pure ocular onset.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committees of Tangdu Hospital, Air Force Medical University (Approval Number: TDLL-KY-202105-04). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SZ, KZ, and JG: conceptualization. XY, JD, KR, JL, and CZ: data curation. SS: formal analysis. JG: funding acquisition, project administration, and writing—review and editing. SZ and XY: investigation. SZ, XY, KZ, and JG: methodology. JD, KR, and JL: resources. ZL: supervision. KZ: validation. SZ, XY, and CZ: visualization. SZ: writing—original draft. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Science and Technology Innovation and Development Foundation of Tangdu Hospital (Grant Number 2019LCYJ010) and the Excellent Personnel Foundation of Tangdu Hospital in 2021.

ACKNOWLEDGMENTS

The authors greatly appreciate Fenying Chen and Tingge Zhu for their excellent contribution to RNS test implementation and result interpretation. The authors would like to thank Kindstar Global for professional assistance in detecting and verifying MG-related antibodies.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.857402/full#supplementary-material

REFERENCES

1. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol.* (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3
2. Petersson M, Feresiadou A, Jons D, Ilinca A, Lundin F, Johansson R, et al. Patient-reported symptom severity in a nationwide myasthenia gravis cohort: cross-sectional analysis of the Swedish GEMG Study. *Neurology.* (2021) 97:e1382–1391. doi: 10.1212/WNL.0000000000012604
3. Fan L, Ma S, Yang Y, Yan Z, Li J, Li Z. Clinical differences of early and late-onset myasthenia gravis in 985 patients. *Neurol Res.* (2019) 41:45–51. doi: 10.1080/01616412.2018.1525121
4. Zivković SA, Clemens PR, Lacomis D. Characteristics of late-onset myasthenia gravis. *J Neurol.* (2012) 259:2167–71. doi: 10.1007/s00415-012-6478-6
5. Sih M, Soliven B, Mathenia N, Jacobsen J, Rezania K. Head-drop: a frequent feature of late-onset myasthenia gravis. *Muscle Nerve.* (2017) 56:441–4. doi: 10.1002/mus.25526

6. Yildiz Celik S, Durmus H, Yilmaz V, Saruhan Direskeneli G, Gulsen Parman Y, Serdaroglu Oflazer P, et al. Late-onset generalized myasthenia gravis: clinical features, treatment, and outcome. *Acta Neurol Belg.* (2020) 120:133–40. doi: 10.1007/s13760-019-01252-x
7. Sakai W, Matsui N, Ishida M, Furukawa T, Miyazaki Y, Fujita K, et al. Late-onset myasthenia gravis is predisposed to become generalized in the elderly. *eNeurologicalSci.* (2016) 2:17–20. doi: 10.1016/j.ensci.2016.02.004
8. Donaldson DH, Ansher M, Horan S, Rutherford RB, Ringel SP. The relationship of age to outcome in myasthenia gravis. *Neurology.* (1990) 40:786–90. doi: 10.1212/WNL.40.5.786
9. Alkhawajah NM, Oger J. Treatment of myasthenia gravis in the aged. *Drugs Aging.* (2015) 32:689–97. doi: 10.1007/s40266-015-0297-2
10. Vijayan J, Menon D, Barnett C, Katzberg H, Lovblom LE, Bril V. Clinical profile and impact of comorbidities in patients with very-late-onset myasthenia gravis. *Muscle Nerve.* (2021) 64:462–6. doi: 10.1002/mus.27369
11. Ding J, Zhao S, Ren K, Dang D, Li H, Wu F, et al. Prediction of generalization of ocular myasthenia gravis under immunosuppressive therapy in Northwest China. *BMC Neurol.* (2020) 20:238. doi: 10.1186/s12883-020-01805-1
12. Li F, Hotter B, Swierzy M, Ismail M, Meisel A, Rückert JC. Generalization after ocular onset in myasthenia gravis: a case series in Germany. *J Neurol.* (2018) 265:2773–82. doi: 10.1007/s00415-018-9056-8
13. Wong SH, Petrie A, Plant GT. Ocular myasthenia gravis: toward a risk of generalization score and sample size calculation for a randomized controlled trial of disease modification. *J Neuroophthalmol.* (2016) 36:252–8. doi: 10.1097/WNO.0000000000000350
14. Feng X, Huan X, Yan C, Song J, Lu J, Zhou L, et al. Adult ocular myasthenia gravis conversion: a single-center retrospective analysis in China. *Eur Neurol.* (2020) 83:182–8. doi: 10.1159/000507853
15. Li M, Ge F, Guo R, Ruan Z, Gao Y, Niu C, et al. Do early prednisolone and other immunosuppressant therapies prevent generalization in ocular myasthenia gravis in Western populations: a systematic review and meta-analysis. *Ther Adv Neurol Disord.* (2019) 12:1756286419876521. doi: 10.1177/1756286419876521
16. Aragonès JM, Bolíbar I, Bonfill X, Bufill E, Mummany A, Alonso F, et al. Myasthenia gravis: a higher than expected incidence in the elderly. *Neurology.* (2003) 60:1024–6. doi: 10.1212/01.WNL.0000050461.05432.C5
17. Somnier FE. Increasing incidence of late-onset anti-AChR antibody-seropositive myasthenia gravis. *Neurology.* (2005) 65:928–30. doi: 10.1212/01.wnl.0000176067.32186.a3
18. Casetta I, Groppo E, De Gennaro R, Cesnik E, Piccolo L, Volpato S, et al. Myasthenia gravis: a changing pattern of incidence. *J Neurol.* (2010) 257:2015–9. doi: 10.1007/s00415-010-5651-z
19. Murai H, Yamashita N, Watanabe M, Nomura Y, Motomura M, Yoshikawa H, et al. Characteristics of myasthenia gravis according to onset-age: Japanese nationwide survey. *J Neurol Sci.* (2011) 305:97–102. doi: 10.1016/j.jns.2011.03.004
20. Barnett C, Bril V. New insights into very-late-onset myasthenia gravis. *Nat Rev Neurol.* (2020) 16:299–300. doi: 10.1038/s41582-020-0345-3
21. Cortés-Vicente E, Álvarez-Velasco R, Segovia S, Paradas C, Casasnovas C, Guerrero-Sola A, et al. Clinical and therapeutic features of myasthenia gravis in adults based on age at onset. *Neurology.* (2020) 94:e1171–80. doi: 10.1212/WNL.0000000000008903
22. Hellmann MA, Mosberg-Galili R, Steiner I. Myasthenia gravis in the elderly. *J Neurol Sci.* (2013) 325:1–5. doi: 10.1016/j.jns.2012.10.028
23. Witoonpanich R, Dejthevaporn C, Sriphrapradang A, Pulkes T. Electrophysiological and immunological study in myasthenia gravis: diagnostic sensitivity and correlation. *Clin Neurophysiol.* (2011) 122:1873–7. doi: 10.1016/j.clinph.2011.02.026
24. Nair AG, Patil-Chhablani P, Venkatramani DV, Gandhi RA. Ocular myasthenia gravis: a review. *Indian J Ophthalmol.* (2014) 62:985–91. doi: 10.4103/0301-4738.145987
25. Vincent A, Clover L, Buckley C, Grimley Evans J, Rothwell PM. UK Myasthenia Gravis Survey. Evidence of underdiagnosis of myasthenia gravis in older people. *J Neurol Neurosurg Psychiatry.* (2003) 74:1105–8. doi: 10.1136/jnnp.74.8.1105
26. Wang L, Zhang Y, He M. Clinical predictors for the prognosis of myasthenia gravis. *BMC Neurol.* (2017) 17:77. doi: 10.1186/s12883-017-0857-7
27. Stacy S, Williams EL, Standifer NE, Pasquali A, Krolick KA, Infante AJ, et al. Maintenance of immune tolerance to a neo-self AChR antigen with aging: implications for late-onset autoimmunity. *J Immunol.* (2010) 184:6067–75. doi: 10.4049/jimmunol.0901618
28. Kuypers DR. Immunotherapy in elderly transplant recipients: a guide to clinically significant drug interactions. *Drugs Aging.* (2009) 26:715–37. doi: 10.2165/11316480-000000000-00000
29. Gurwitz JH, Field TS, Harrold LR, Rothschild J, Debellis K, Seger AC, et al. Incidence and preventability of adverse drug events among older persons in the ambulatory setting. *JAMA.* (2003) 289:1107–116. doi: 10.1001/jama.289.9.1107
30. Alshekhlee A, Miles JD, Katirji B, Preston DC, Kaminski HJ. Incidence and mortality rates of myasthenia gravis and myasthenic crisis in US hospitals. *Neurology.* (2009) 72:1548–54. doi: 10.1212/WNL.0b013e3181a41211
31. Antonini G, Morino S, Gragnani F, Fiorelli M. Myasthenia gravis in the elderly: a hospital based study. *Acta Neurol Scand.* (1996) 93:260–2. doi: 10.1111/j.1600-0404.1996.tb00517.x
32. Slesak G, Melms A, Gerneth F, Sommer N, Weissert R, Dichgans J. Late-onset myasthenia gravis. Follow-up of 113 patients diagnosed after age 60. *Ann N Y Acad Sci.* (1998) 841:777–80. doi: 10.1111/j.1749-6632.1998.tb11017.x
33. Alkhawajah NM, Oger J. Late-onset myasthenia gravis: a review when incidence in older adults keeps increasing. *Muscle Nerve.* (2013) 48:705–10. doi: 10.1002/mus.23964

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Neurology

REVIEW
published: 25 April 2022
doi: 10.3389/fneur.2022.886625

Corticosteroid Treatment-Resistance in Myasthenia Gravis

Henry J. Kaminski and Jordan Denk*

Department of Neurology and Rehabilitation Medicine, George Washington University, Washington, DC, United States

Chronic, high-dose, oral prednisone has been the mainstay of myasthenia gravis treatment for decades and has proven to be highly beneficial in many, toxic in some way to all, and not effective in a significant minority. No patient characteristics or biomarkers are predictive of treatment response leading to many patients suffering adverse effects with no benefit. Presently, measurements of treatment response, whether taken from clinician or patient perspective, are appreciated to be limited by lack of good correlation, which then complicates correlation to biological measures. Treatment response may be limited because disease mechanisms are not influenced by corticosteroids, limits on dosage because of adverse effects, or individual differences in corticosteroids. This review evaluates potential mechanisms that underlie lack of response to glucocorticoids in patients with myasthenia gravis.

Keywords: myasthenia gravis, corticosteroids, lymphocytes, biomarkers, clinical outcome measures

OPEN ACCESS

Edited by:
Hai-Feng Li,
Capital Medical University, China

Reviewed by:
Sarah Hoffmann,
Charité Universitätsmedizin Berlin, Germany
Kimiaki Utsugisawa,
Hanamaki General Hospital, Japan

****Correspondence:***
Henry J. Kaminski
HKaminski@mfa.gwu.edu

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *28 February 2022*
Accepted: *21 March 2022*
Published: *25 April 2022*

Citation:
Kaminski HJ and Denk J (2022) Corticosteroid Treatment-Resistance in Myasthenia Gravis. Front. Neurol. 13:886625. doi: 10.3389/fneur.2022.886625

INTRODUCTION

Glucocorticoids (GC) are simultaneously the best and worst medications for patients with myasthenia gravis (MG). Their efficacy cannot be denied based on decades of use in clinical practice and an extensive evidence base of retrospective studies, expert opinion, and several consensus guidelines as well as a limited number of randomized trials (1–8). In 1948 ACTH was first used for MG treatment and many reports in the following two decades appreciated a therapeutic benefit (9, 10). Chronic prednisone treatment over months to years became the standard of care during the 1970's (11). Short high-dose treatment with methylprednisolone has been used (12–14). However, the usefulness of GCs is diminished by their significant adverse effects. The need to reduce corticosteroid exposure has led to the use of immunosuppressives, plasma exchange, intravenous immunoglobulin, and more recently a number of biologics for MG treatment (15, 16). The balance of effectiveness and adverse effects has led to the reduction of overall prednisone dose as a measure of efficacy in some clinical trials (17–21).

Regardless of the specific GC preparation and dosing regimen, there is a core of patients with MG who have a poor clinical response. Two large cross sectional studies of patients with MG indicated that there was a group of patients not achieving a minimal manifestation status despite higher prednisone dosage (16). Thus far, there are no patient characteristics that predict treatment-resistance (6). Shared with MG are most inflammatory or autoimmune conditions with a core of 20–30% of patients who do not improve with GC treatment (22, 23).

This review will broadly assess potential mechanisms that limit treatment response to GC in MG.

THE CHALLENGE OF DEFINING TREATMENT RESPONSE

A significant challenge for MG and many disorders is the lack of reliable, objective markers of disease activity. This is in marked contrast, for example, to autoimmune thrombolytic anemia in which platelet counts track with severity of disease manifestations, respiratory parameters for asthma, or gadolinium enhancing lesions identified by magnetic resonance imaging in multiple sclerosis. Often disease severity is assessed by response to a treatment; however, this approaches a circular argument. If a drug does not work, it may simply not be targeting disease mechanisms, not accessing the site of pathology, or achieving appropriate levels to influence the disease. None of these suggest that the underlying disease mechanisms themselves are "more severe".

Treatment resistance may stem from three broad, and potentially overlapping, reasons: (1) GC may not impact fundamental disease mechanisms, (2) excess susceptibility to corticosteroid adverse effects, which compromise ability to achieve therapeutic doses, and (3) phenotypic variations among patients that limit biological response to the GC. All these may be difficult to differentiate if severity of disease is defined as a lack of response to GC. For MG, treatment response has been assessed from various perspectives. Clinical outcome measures for MG have evolved from simple physician-centric determination of improvement to standardized strength assessment performed by trained individuals to patient reported outcomes (24, 25). Primary outcome measures for randomized trials in MG have included the total dose of GC over time, the quantitative MG Score, and the MG-Activities of Daily Living with the last of which has become the primary measure recommended by the FDA for drug approval. There has been an assumption that improvement in standardized assessments of muscle strength, as done in the QMG, would equate to improvement in patient reported outcomes, but this is not the case as appreciated by the relatively poor concordance of clinical outcome measures (26, 27). The explanation for this discrepancy lies in the complex interaction of the measurement used, disease pathology, treatment used with its adverse effects, and the individual response to disease, which includes social determinants of health and a person's personality traits. The expectation that circulating autoantibodies would be a surrogate for treatment response has not proven true. The acetylcholine receptor antibody level does not correlate with improvement (28) and the rate of change of antibody correlates only roughly (29). Small studies support muscle specific kinase (MuSK) antibodies associate with treatment response, but this has not been rigorously evaluated (30, 31). The decremental response with repetitive stimulation and abnormalities of the single fiber evaluation also do not correlate well enough with clinical disease severity to be used as a surrogate biomarker (17, 32).

GLUCOCORTICOID MECHANISMS OF ACTION

Cortisol, the endogenous glucocorticoid, is synthesized and released by the adrenal glands as regulated by the hypothalamic-pituitary-adrenal (HPA) axis (**Figure 1**). Corticotrophin-releasing hormone (CRH) from the hypothalamus activates corticotrophic cells of the pituitary leading to release of adrenal corticotropic hormone (ACTH), which then acts to enhance synthesis and release of cortisol from the adrenal cortex. Blood cortisol levels follow a circadian rhythm with an early morning peak and a nighttime nadir (33), and increase in response to stress including emotional reactions, physical challenges, and tissue trauma (23, 34). These diurnal fluctuations also impact the immune system and likely influence immune reactions to outside stimuli [infections) and by extension autoimmune reactions (33). The HPA axis employs a negative feedback system that occurs at both the levels of the hypothalamus and the anterior pituitary gland to moderate continued release in states of GC excess. Additionally, the hypothalamus can be stimulated by cytokine activation *via* interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-6 (35) as would occur in inflammatory and autoimmune diseases. Psychological stress also increases GC production due to increased noradrenaline levels, which further stimulate CRH and cause an increase in pro-inflammatory cytokines, all of which stimulate the HPA axis (33).

Cortisol binds the carrier protein, corticosteroid-binding globulin (CBG), for its distribution *via* the circulation. Bound cortisol is inactive, and only the small fraction of unbound GC, which is lipophilic, diffuses readily across cell membranes. Cytoplasmic cortisol binds to the GC receptor (GCR). The bound GC and GCR impact biological processes through (1) activity as a transcription factor binding to GC response elements of numerous genes, (2) interactions with other transcription factors including nuclear factor-κB (NF-κB) and activator protein 1 (AP-1), and (3) repression of gene transcription through binding of inhibitory GC response elements and binding of other transcription factors to prevent their action (35). GCs have been estimated to impact expression of 20% of the genome (36). GCs also act through non-genomic mechanisms. The lipophilic properties of GCs lead to their ability, in the absence of the glucocorticoid receptor (GR), to enter lipid membrane, which alters membrane fluidity and interaction with membrane bound proteins, including ion channels. The alteration of sodium and calcium transfer appears to be a factor in mediating some anti-inflammatory effects. To add to the complexity of GC influences each cell differs in the nature of transcriptional factors and other proteins for the GC to interact. Given their numerous tissue targets, excess glucocorticoid states, whether endogenous as in Cushing's syndrome or exogenous provided as prednisone, can lead to numerous adverse effects with wide inter-individual variation for treatment response. Synthetic GC, i.e., prednisone and dexamethasone, are not subject to endogenous inhibitors of cortisol activity making them more potent anti-inflammatory agents. Prednisone binds the GCR with higher affinity and mineralocorticoid receptors with lower

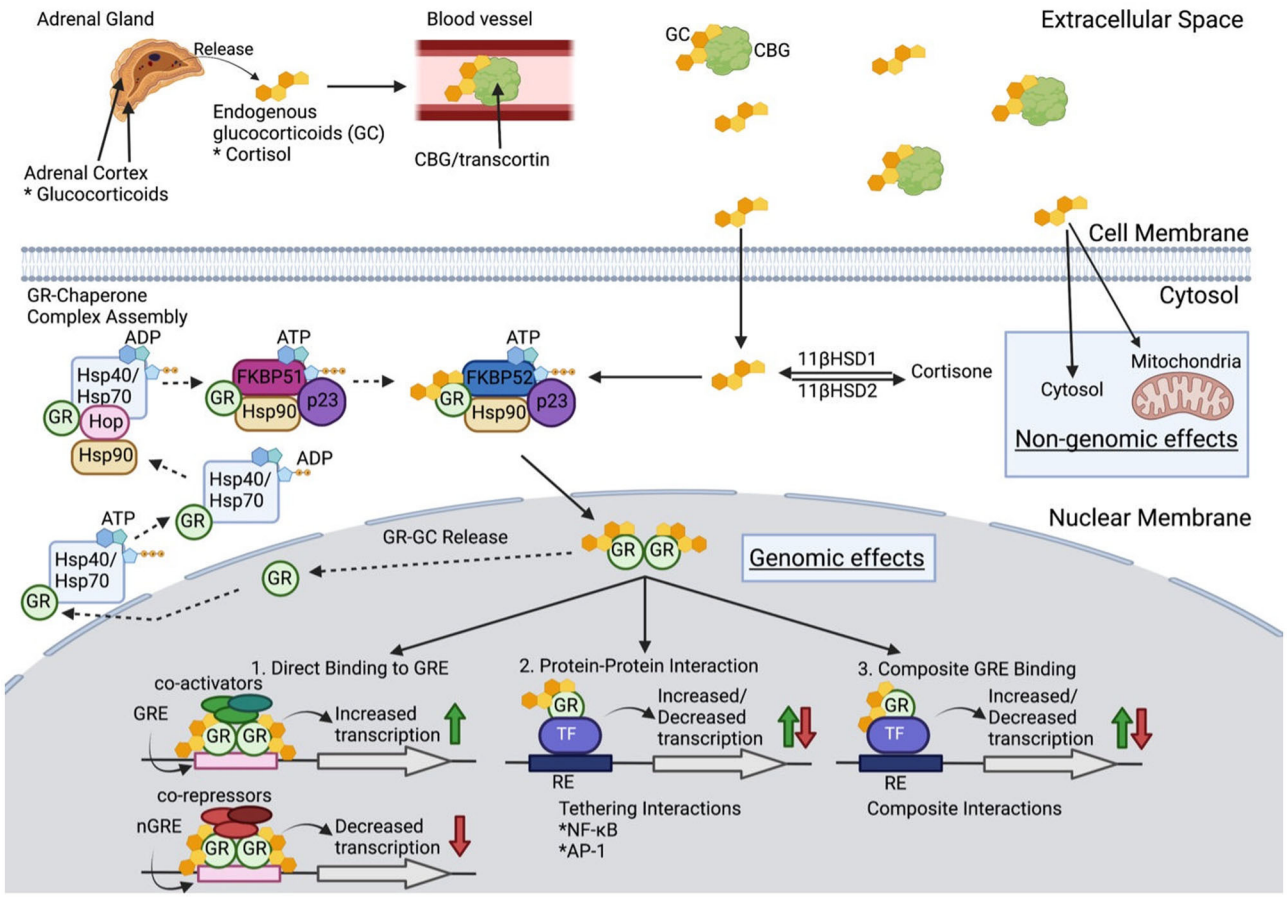


FIGURE 1 | Glucocorticoid Molecular Physiology. Once released from the adrenal cortex, glucocorticoids (GC) travel through blood with the carrier protein, corticosteroid-binding globulin (CBG). Only 5% of extracellular GCs remain bioactive after binding to CBG. GC diffuse through the cell membrane to either (1) be converted into inactive cortisone *via* 11β-hydroxysteroid dehydrogenase 2, (2) have non-genomic effects in the cytosol or mitochondria, or (3) bind to the glucocorticoid receptor (GCR) as a chaperone complex to later exert genomic effects in the nucleus. When no cytoplasmic bioactive GCs are present, a multiprotein complex begins GR maturation to prepare for GC binding. Once matured, GCR's two nuclear localizations signals are exposed, which are then bound by nucleoporin and importins that translocate cytoplasmic GC into the nuclear membrane. Inside the nucleus, the GCR complex can be released, and the GR can be transported back to the cytoplasm, or the GR-GC complex can exert its function. Genomic effects include three categories: (1) direct binding to GC response elements (GREs) or negative GREs (nGREs) which recruit transcriptional co-activators and co-repressors respectively, (2) protein-protein interaction with transcription factors (TF) that modify transcription, and (3) composite interactions that involve DNA binding to GRE to alter transcription (see text for further details).

affinity than does cortisol, thereby limiting mineralocorticoid-based complications.

The GCR is key in mediating many of the actions of GC. The protein has three functional regions. (1) The constitutively active ligand-independent activation domain (AF-1) is located in the N-terminal region and is bound by the transcriptional machinery and coregulators. (2) The DNA-binding domain allows for binding of the GR to DNA and regulatory proteins. (3) Ligand-binding domain of the C-terminus also serves to interact with other transcriptional proteins, chaperone proteins, and coregulators. The GR protein activity is subject to regulation by phosphorylation, ubiquitination, and acetylation. The human GR gene transcript undergoes alternative splicing to generate GRα and GRβ isoforms, each with specific activity. The isoforms are nearly identical through amino acid 727, but GRα contains an additional 50 amino acids, and GRβ differs with an additional 15 non-homologous amino acids. GRβ is present in the nucleus and is transcriptionally active with the capability to repress or activate genes regulated by GRα. GRβ can inhibit GRα activity. Proinflammatory cytokines and other signals increase the expression of GRβ and mediate GC resistance (37). Other GCR isoforms exist but are less well understood and have not been associated with GC resistance. There are an increasing number of proteins being identified, which bind the GCR and its complex with GC and are likely to influence GC activity. A detailed discussion of these is beyond the scope of this review and reader should see the excellent summary of Petta et al. (35).

The GCR suppresses pro-inflammatory pathways supported by NF-κB, AP-1, and MAPK (23). Each of these major pathways influence cell survival, apoptosis, proliferation, differentiation

and production of activate cytokines, chemokines, and other key aspects of inflammation. Although all have predominant pro-inflammatory, a chronic high level of NF-κB activity may lead not only to chronic inflammation, but also to GC resistance by blocking the GCR signaling pathway. Such chronic low level inflammation has been implicated in the pathogenesis of many diseases (38). GC also has *pro-inflammatory* effects in certain situations including the dose of GC and timing during the development of inflammation (39). For example, low dose GC will enhance delayed-hypersensitivity in rat models, but their chronic, high dose administration will enhance the response (40).

GC have significant influences on cellular immunity. GC inhibit dendritic cell maturation through reduction of expression of MHC class II and costimulatory molecules. They also have complicated effects on T cells, which include interference in TCR signaling leading to reduced T cell activity, but GC appears to have a suppressive effect on Th1 and Th17 cells, but promote Th2 and Treg cells. GC treatment increases frequencies of circulating Treg cells, which is likely GC mediated increase in forkhead box P3 (FOXP3) through upregulation of GILZ87 (23). Thymocytes are particularly sensitive to GC-mediated apoptosis. The details of GC effects on B cells is being elucidated. GC treatment reduces antibody concentrations in circulation and immature B cells, which express GCR, are particularly sensitive to induced apoptosis in contrast to more mature B cells and plasm cells. However, emerging literature supports that GC can have pro-inflammatory effects. GC enhances sensitivity of some cytokine receptors, while reducing circulating levels of these cytokines. Expression profiling indicates that gene expression of innate immunity including complement components, receptors of chemokines and cytokines, are upregulated, while T cell pathway genes are increased. Cain and Cidlowski propose that in the normal condition immune cells are sensitized to detected infections and other harmful signals leading to tissue damage and thereby the immune system can react rapidly (23). In a pro-inflammatory state, stress-induced increases in cortisol or exogenous GC will reduce the acute immune response. This dual state of pro- and anti-inflammatory effects leads to the complicated effects of exogenous GC treatments in autoimmune diseases and the impact of GC dosage and duration of treatment.

STEROID-RESISTANCE IN MYASTHENIA GRAVIS

A detailed analysis of MG pathophysiology is beyond the scope of this discussion but are reviewed in the context of treatment resistance. The authors recommend readers see a recent review by Huijbers et al. (41). As mentioned above, there are three categories of explanation why patients with MG would not respond to GC treatment. The disease-causing mechanisms are not influenced by GC, the adverse effects of GC are not tolerated leading to an inadequate dose, or there are individual traits which limit the effect of GC treatment.

Underlying Pathology Does Not Respond to Corticosteroids

Among the best examples of apparently similar inflammatory diseases with contrasting responses to GC treatment are inflammatory pulmonary conditions, which account for about 60% of prescriptions for oral GC in the United Kingdom (42). Asthma, chronic obstructive pulmonary disease, interstitial pulmonary fibrosis, and cystic fibrosis demonstrate inflammatory infiltrates (43) in the lung with an expectation that GC therapy would moderate the severity of each disease, but a significant benefit is only appreciated in patients with asthma.

As an autoimmune disease with a preponderance of patients improving with GC treatment, there appears to be no a priori reason for GC to be unable to target the immunopathology of MG. However, the possibility that some mechanisms driving pathology, which are not amenable to GC treatment should not be discounted. MG is not a single disease, but rather has subgroups defined by age, thymic pathology and autoantibody status. Existing data supports MuSK MG being primarily a disease of short-lived plasma cells, which are more sensitive to GC treatment, compared to long-lived plasma cells of AChR antibody positive MG (31, 44). The better response to anti-CD20 treatment of MuSK MG than AChR MG supports that short-lived CD20 expressing plasma cells are critical in disease pathology compared to long-lived plasma cells, which do not express CD20 (45, 46). GC-resistance may change over time with the potential for long-lived plasma cells becoming the major driver of pathology, compared to earlier in the disease may also induce resistance itself. Other than plasma cell lineage factors disease factors, which are not amenable to GC sensitivity are not known.

Adverse Effect Susceptibility

One aspect of GC resistance, which should not be overlooked, is the variation in susceptibility to adverse effects, which then compromises ability to achieve therapeutic doses. Despite the well-appreciated adverse effects of GC treatment, there is limited data on the inter-individual susceptibility to adverse effects. Upwards of one to two thirds of patients with MG hav e adverse effects related to GC therapy (19, 47). The major risk factor for GC morbidity is the cumulative dose of GC, but even with lower dose regimens of 20–30 mg of prednisone vs. the historical standards 60–80 mg per day dosing, intolerable adverse effects occur (16, 48, 49). The most common adverse effects are weight gain, Cushingoid appearance, and skin changes including acne, while more medically severe effects, but rare complications, include gastric and esophageal irritation, compression fractures, and aseptic necrosis of the femoral head. Between these ends of severity are worsening hypertension, diabetes, glaucoma and cataract formation. Poorly-documented adverse effects, which occur in essentially all patients, are insomnia and mood changes from irritability and various degrees of depression. A study of over a thousand rheumatoid arthritis patients found a dose-dependent relation with Cushingoid features, peripheral edema, skin bruising and threshold effect of 7.5 mg per day with glaucoma, depression and hypertension, while even five

mg per day increased incidence of weight gain and even a lower dose of cataract formation (50). McDowell et al. evaluated adverse effects in a population of patients with severe asthma and using a quantitative instrument specific for GC treatment complications confirmed significant inter-individual variability in adverse effects, which is consistent with the long-standing clinical impression. The inter-individual susceptibility to adverse effects and treatment resistance are intertwined from the clinician and patient perspective but biological mechanisms that drive improvement vs. complications are likely distinct. GC differentially influence gene expression of pathways, which moderate inflammatory and adverse effects (51) with adverse effects primarily associated with the transactivation of genes by the corticosteroid, which has led to attempts to engineer compounds that support suppression of pro-inflammatory gene transcription, but limit transactivation (52–54).

Sensitivity to Treatment Effect of Glucocorticoids

As should be clear from the summary of GC action, there is the potential for GC efficacy to be compromised at many steps from administration to final effector mechanisms. Below we review the presently known mechanisms of GC resistance that may impact efficacy for MG.

GC Metabolism

Despite decades of use, there is relatively poor characterization of the impact of GC metabolism on therapeutic benefit. Efficacy properties of any drug begin with its pharmacokinetic profile. Exogenous GC are not subject to endogenous moderators of cortisol (55). Prednisone and prednisolone are the most frequently used GC in treatment of MG with both drugs rapidly absorbed after oral ingestion. Prednisone is converted to prednisolone rapidly by the action of 11β-hydroxysteroid dehydrogenase with a peak blood concentration within 3 h. High inter-individual difference in bioavailability of prednisone has been documented (56). Prednisone is cleared primarily by hepatic metabolism by the P450 system and drugs, which block or enhance P450 enzymes will modify the half-life of the drug. Prednisone itself may modify xenobiotic pathways that metabolize the drug, which further enhances the complexity of inter-individual variation of efficacy (57). In addition, both the GCR and xenobiotic receptor activation inhibit the activity of NF-κB, a master regulator of the immune response (58). Also, NF-κB activation reciprocally inhibits xenobiotic metabolism, creating a complex feedback loop. The simple variation of metabolism of prednisone could impact its efficacy in individual patients with MG. Genetic differences in drug metabolism are being appreciated but have not yet reached an understanding to guide GC therapy.

Pharmacogenetics and Glucocorticoid Resistance

Genetic variations are well-appreciated to influence drug responses or adverse effects to GC but have yet to be defined well enough to guide practice. Polymorphisms in the GCR gene are associated with response to GC in ulcerative colitis and rheumatoid arthritis (59–61), and we also found this to be the case in GC treatment response in patients with MG (62) (**Table 1**). The only other gene with genetic polymorphisms associated with treatment response in MG is osteopontin (63). Circulating GRβ levels have been found to be associated with GC resistance in rheumatoid arthritis, SLE, and asthma (64–66). Hypomethylation of NLRP3 gene promoter discriminates glucocorticoid-resistant from GC-sensitive idiopathic nephrotic syndrome patients (67). P53 interacts with GR to promote anti-inflammatory pathways and patients with rheumatoid arthritis who did not respond to GC treatment showed reduced p53 expression levels in blood mononuclear cells (68). Genetic variations, including ones that vary in significance based on sex, are increasingly being appreciated in response to GC therapy but have yet to guide treatment decisions. The response to GC therapy may wane over time appreciated for some conditions is produced by a downregulation of the GRα (69).

Lymphocyte Sensitivity

Investigations of cultured lymphocytes of patients with rheumatoid arthritis, inflammatory bowel diseases and systemic lupus demonstrate a sensitivity to *in vitro* lysis when cultured with GC, which correlates with the clinical benefit observed in these patients (42, 70, 71). Of note, the *in vitro* sensitivity is observed in non-disease control subjects and therefore is not a function of disease activity. Studies of African Americans with asthma show less *in vitro* sensitivity to GC, which again correlates with poorer clinical response to GC therapy (72). Glycosphingolipid metabolism, urea cycle, and pentose phosphate pathways are associated with *in vitro* glucocorticoid resistance in pregnant African American women (73). Differences in transcription of NF-κB and other genes are associated with the degree of lymphocyte sensitivity to glucocorticoids (70, 74).

Sex and Gender Differences in Autoimmunity and Glucocorticoid Resistance

Sex refers to characteristics specific to biologically determined properties of the sex chromosomes. Gender encompasses

TABLE 1 | Examples of genes with single nucleotide polymorphisms associated with GC resistance.

Gene	Protein	Disease GC Resistance
NR3C1	Glucocorticoid Receptor	MG, pediatric nephrotic syndrome
FKBP5	FK506 binding protein 5	Inflammatory bowel disease
IL-4	Interleukin-4	Nephrotic syndrome
IL-6	Interleukin-6	Nephrotic syndrome
MIF	macrophage migration inhibitory factor	inflammatory bowel disease, rheumatoid arthritis
GLCCI1	Glucocorticoid Induced 1	Asthma
MDR1	P-glycoprotein	Nephrotic syndrome, inflammatory bowel disease, rheumatoid arthritis
NR1I2	Pregnane X receptor	Nephrotic syndrome

biological differences coupled with social and cultural factors, which define women and men. Under the age of 40 years about two thirds of patients with MG are women while with advancing age the gender discrepancy begins to shift toward men. Rheumatoid arthritis and multiple sclerosis share a similar distribution, in contrast, women account for over 90% of cases of SLE and Sjogren's. These observations support that there are fundamental gender differences in susceptibility to initiation and maintenance of autoimmune disorders that are dependent on the specific disease. There is an ever-increasing appreciation of the differences in the immune responses of females and males, which span species from Drosophila to humans. Females develop more intense innate and adaptive immune reactions than males allowing for better clearance of infectious agents as well as greater responses to vaccinations; however, this comes at the price of greater susceptibility to autoimmune process (75, 76). Sex hormones and immune system related genes on the X chromosome hosts are factors, which drive these differences. The impact of sex hormones on autoimmunity is illustrated by the general observation that disease severity is reduced during pregnancy and exacerbate post-partum. Pregnancy also leads to the transmission of fetal cells to the mother and these foreign cells can persist for decades. Maternal cells also persist in individuals at very low levels throughout postnatal development. The maternal receipt of fetal cells likely expands immune tolerance in the mother during pregnancy, but they may also contribute to increased risk of autoimmune disease in women of child bearing years (77). Epigenetic factors impact gene expression on the X chromosome and thereby provide mechanisms on how the environment may shape gender differences in autoimmunity (78–80).

The severity of autoimmune diseases vary based on gender. Men with psoriasis, multiple sclerosis and SLE have a worse prognosis, in contrast to there not being a difference in rheumatoid arthritis (79). Young women also have a poorer response compared to men to GC therapy for inflammatory bowel disease (81). Mortality rates generally are higher among women with autoimmune diseases, but this data is difficult to interpret as to whether biological, social, comorbidities, or other factors drive these observations. A patient reported registry study indicated that women with MG have a poorer quality of life (82), but there is limited data as to whether women respond less well to treatment. Women report a poorer response to overall treatments for MG and have greater adverse effects from prednisone (82, 83). Endogenous and exogenous GC influence gene expression, including those of the immune system, in a sex specific manner (84).

CLINICAL CONSEQUENCES

Identifying treatment-resistant patients prior to initiation of GC is presently not possible and therefore, the clinician needs to be proactive in discontinuation of prednisone treatment to prevent greater adverse effects than can be balanced by benefit. Consensus guidelines recommend moving to alternative therapies when initial GC therapy at "adequate" dosing does not improve or worsens the patient's condition or if adverse effects are deemed intolerable by patient or physician (8). The consensus guideline provides options of slow, alternate dose escalation or a high-dose rapid induction. No specific time-frame for improvement, level of response, or severity of adverse effects is defined. A responsive patient to prednisone usually does so in 4–6 weeks after prednisone initiation. This may not be complete, but physician and patient should expect a situation close to minimal manifestations. Again, both patient and clinician should guard against being content with significant improvement from a poor baseline and accepting disability.

The MG community is blessed with therapeutic options for GC treatment resistance, which are detailed in a number of recent reviews (15, 21). For all AChR-Ab positive patients under 65 years of age as was defined in the MGTX study this would mean a thymectomy regardless of response to prednisone (19). Relatively, rapidly acting approaches as intravenous immunoglobulin, plasma exchange, complement inhibition or FcRn blockers, should be used for patients presenting with significant disability and an initial poor response to prednisone. However, none of these treatments will lead to remission and therefore, for long-term reduction of antibody producing cells, immunosuppressives and B cell ablation therapy should be considered. Tapering of prednisone should begin with initiation of additional therapies and its speed dependent on the nature of the additional treatment and the expected onset of action.

CONCLUDING REMARKS

MG therapeutic development is making incredible advances (21) with agents that specifically target effector mechanisms as well as autoantibody producing cells and attempts to reestablish tolerance. Despite the new drugs approved, and ones on the horizon, GC treatment will continue to be the primary therapy used for MG care for the foreseeable future (8). Detailed investigation of patients who demonstrate differential responses to GC offer a powerful set of experiments to understand MG mechanisms and further define differences, which will allow development of personalized medicine for patients. The application of broad spectrum proteomic, genomic, metabolomic, and microbiome approaches linked to precise clinical characterization will be key to elucidating subtle differences in disease mechanisms and treatment response.

AUTHOR CONTRIBUTIONS

HK formulated review, wrote the majority of the manuscript, and reviewed the entire final manuscript. JD drafted portions of the text, developed the figure and legend, and reviewed the final submission. Both authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the MGNet a member of the Rare Disease Clinical Research Network Consortium (RDCRN) NIH U54 NS115054. Funding support for the DMCC was provided by the National Center for Advancing Translational Sciences (NCATS) and the National Institute of Neurological Disorders and Stroke (NINDS).

REFERENCES

1. Pascuzzi RM, Coslett HB, Johns TR. Long-term corticosteroid treatment of myasthenia gravis: report of 116 patients. *Ann Neurol.* (1984) 15:291–8. doi: 10.1002/ana.410150316
2. Cosi V, Citterio A, Lombardi M, Piccolo G, Romani A, Erbetta A. Effectiveness of steroid treatment in myasthenia gravis: a retrospective study. *Acta Neurol Scand.* (1991) 84:33–9. doi: 10.1111/j.1600-0404.1991.tb04899.x
3. Evoli A, Batocchi AP, Palmisani MT, Lo Monaco M, Tonali P. Long-term results of corticosteroid therapy in patients with myasthenia gravis. *Eur Neurol.* (1992) 32:37–43. doi: 10.1159/000116785
4. Schneider-Gold C, Gajdos P, Toyka KV, Hohlfeld RR. Corticosteroids for myasthenia gravis. *Cochrane Database Syst Rev.* (2005) CD002828. doi: 10.1002/14651858.CD002828.pub2
5. Hoffmann S, Kohler S, Ziegler A, Meisel A. Glucocorticoids in myasthenia gravis - if, when, how, and how much? *Acta Neurol Scand.* (2014) 130:211–21. doi: 10.1111/ane.12261
6. Imai T, Suzuki S, Tsuda E, Nagane Y, Murai H, Masuda M, et al. Oral corticosteroid therapy and present disease status in myasthenia gravis. *Muscle Nerve.* (2015) 51:692–6. doi: 10.1002/mus.24438
7. Benatar M, Mcdermott MP, Sanders DB, Wolfe GI, Barohn RJ, Nowak RJ, et al. Efficacy of prednisone for the treatment of ocular myasthenia (EPITOME): a randomized, controlled trial. *Muscle Nerve.* (2016) 53:363–9. doi: 10.1002/mus.24769
8. Narayanaswami P, Sanders DB, Wolfe G, Benatar M, Cea G, Evoli A, et al. International consensus guidance for management of myasthenia gravis: 2020 update. *Neurology.* (2021) 96:114–22. doi: 10.1212/WNL.0000000000011124
9. Namba T, Shapiro MS, Brunner NG, Grob D. Corticotropin therapy in myasthenia gravis: indications and limitations. *Neurology.* (1970) 20:385.
10. Namba T, Brunner NG, Shapiro MS, Grob D. Corticotropin therapy in myasthenia gravis: effects, indications, and limitations. *Neurology.* (1971) 21:1008–18. doi: 10.1212/WNL.21.10.1008
11. Warmolts JR, Engel WK. Benefit from alternate-day prednisone in myasthenia gravis. *N Engl J Med.* (1972) 286:17–20. doi: 10.1056/NEJM197201062860104
12. Arsura E, Brunner N, Namba T, Grob D. High-dose intravenous methylprednisolone in myasthenia gravis. *Arch Neurol.* (1985) 42:1149–53. doi: 10.1001/archneur.1985.04060110031011
13. Lindberg C, Andersen O, Lefvert AK. Treatment of myasthenia gravis with methylprednisolone pulse: a double blind study. *Acta Neurol Scand.* (1998) 97:370–3. doi: 10.1111/j.1600-0404.1998.tb05968.x
14. Ozawa Y, Uzawa A, Kanai T, Oda F, Yasuda M, Kawaguchi N, et al. Efficacy of high-dose intravenous methylprednisolone therapy for ocular myasthenia gravis. *J Neurol Sci.* (2019) 402:12–5. doi: 10.1016/j.jns.2019.05.003
15. Alabbad S, Algaeed M, Sikorski P, Kaminski HJ. monoclonal antibody-based therapies for myasthenia gravis. *BioDrugs.* (2020) 34:557–66. doi: 10.1007/s40259-020-00443-w
16. Imai T, Suzuki S, Nagane Y, Uzawa A, Murai H, Utsugisawa K. Reappraisal of oral steroid therapy for myasthenia gravis. *Front Neurol.* (2020) 11:868. doi: 10.3389/fneur.2020.00868
17. Benatar M, Sanders DB, Burns TM, Cutter GR, Guptill JT, Baggi F, et al. Recommendations for myasthenia gravis clinical trials. *Muscle Nerve.* (2012) 45:909–17. doi: 10.1002/mus.23330
18. Pasnoor M, He J, Herbelin L, Burns TM, Nations S, Bril V, et al. A randomized controlled trial of methotrexate for patients with generalized myasthenia gravis. *Neurology.* (2016) 87:57–64. doi: 10.1212/WNL.0000000000002795
19. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo HC, Marx A, et al. Randomized trial of thymectomy in myasthenia gravis. *N Engl J Med.* (2016) 375:511–22. doi: 10.1056/NEJMc1611704
20. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo HC, Marx A, et al. Long-term effect of thymectomy plus prednisone versus prednisone alone in patients with non-thymomatous myasthenia gravis: 2-year extension of the MGTX randomised trial. *Lancet Neurol.* (2019) 18:259–68. doi: 10.1016/S1474-4422(18)30392-2
21. Verschuuren JJ, Palace J, Murai H, Tannemaat MR, Kaminski HJ, Bril V. Advances and ongoing research in the treatment of autoimmune neuromuscular junction disorders. *Lancet Neurol.* (2022) 21:189–202. doi: 10.1016/S1474-4422(21)00463-4
22. Quax RA, Manenschijn L, Koper JW, Hazes JM, Lamberts SW, Van Rossum EF, et al. Glucocorticoid sensitivity in health and disease. *Nat Rev Endocrinol.* (2013) 9:670–86. doi: 10.1038/nrendo.2013.183
23. Cain DW, Cidlowski JA. Immune regulation by glucocorticoids. *Nat Rev Immunol.* (2017) 17:233–47. doi: 10.1038/nri.2017.1
24. Kaminski HJ, Alnosair E, Algahtani RM. Clinical trials for myasthenia gravis: a historical perspective. *Ann N Y Acad Sci.* (2018) 1413:5–10. doi: 10.1111/nyas.13545
25. Thomsen JLS, Andersen H. Outcome measures in clinical trials of patients with myasthenia gravis. *Front Neurol.* (2020) 11:596382. doi: 10.3389/fneur.2020.596382
26. Barnett C, Herbelin L, Dimachkie MM, Barohn RJ. Measuring clinical treatment response in myasthenia gravis. *Neurol Clin.* (2018) 36:339–53. doi: 10.1016/j.ncl.2018.01.006
27. Mcpherson T, Aban I, Duda PW, Farzaneh-Far R, Wolfe GI, Kaminski HJ, et al. Correlation of quantitative myasthenia gravis and myasthenia gravis activities of daily living scales in the MGTX study. *Muscle Nerve.* (2020) 62:261–6. doi: 10.1002/mus.26910
28. Sanders DB, Burns TM, Cutter GR, Massey JM, Juel VC, Hobson-Webb L, et al. Does change in AChR antibody level correlate with clinical change in MG? *Muscle Nerve.* (2014) 49:483–6. doi: 10.1002/mus.23944
29. Kojima Y, Uzawa A, Ozawa Y, Yasuda M, Onishi Y, Akamine H, et al. Rate of change in acetylcholine receptor antibody levels predicts myasthenia gravis outcome. *J Neurol Neurosurg Psychiatry.* (2021) 92:963–8. doi: 10.1136/jnnp-2020-325511
30. Bartoccioni E, Scuderi F, Minicuci GM, Marino M, Ciaraffa F, Evoli A. Anti-MuSK antibodies: correlation with myasthenia gravis severity. *Neurology.* (2006) 67:505–7. doi: 10.1212/01.wnl.0000228225.23349.5d
31. Marino M, Basile U, Spagni G, Napodano C, Iorio R, Gulli F, et al. Long-lasting rituximab-induced reduction of specific-but not total-IgG4 in MuSK-positive myasthenia gravis. *Front Immunol.* (2020) 11:613. doi: 10.3389/fimmu.2020.00613
32. Benatar M. A systematic review of diagnostic studies in myasthenia gravis. *Neuromuscul Disord.* (2006) 16:459–67. doi: 10.1016/j.nmd.2006.05.006
33. Shimba A, Ejima A, Ikuta K. Pleiotropic effects of glucocorticoids on the immune system in circadian rhythm and stress. *Front Immunol.* (2021) 12:706951. doi: 10.3389/fimmu.2021.706951
34. Ramamoorthy S, Cidlowski JA. Corticosteroids: mechanisms of action in health and disease. *Rheum Dis Clin North Am.* (2016) 42:15–31. doi: 10.1016/j.rdc.2015.08.002
35. Petta I, Dejager L, Ballegeer M, Lievens S, Tavernier J, De Bosscher K, et al. The interactome of the glucocorticoid receptor and its influence on the actions of glucocorticoids in combatting inflammatory and infectious diseases. *Microbiol Mol Biol Rev.* (2016) 80:495–522. doi: 10.1128/MMBR.00064-15
36. Galon J, Franchimont D, Hiroi N, Frey G, Boettner A, Ehrhart-Bornstein M, et al. Gene profiling reveals unknown enhancing and suppressive actions of glucocorticoids on immune cells. *FASEB J.* (2002) 16:61–71. doi: 10.1096/fj.01-0245com
37. Webster JC, Oakley RH, Jewell CM, Cidlowski JA. Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: a mechanism for the generation of glucocorticoid resistance. *Proc Natl Acad Sci USA.* (2001) 98:6865–70. doi: 10.1073/pnas.121455098

38. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. *Nat Med.* (2019) 25:1822–32. doi: 10.1038/s41591-019-0675-0
39. Cruz-Topete D, Cidlowski JA. One hormone, two actions: anti- and pro-inflammatory effects of glucocorticoids. *Neuroimmunomodulation.* (2015) 22:20–32. doi: 10.1159/000362724
40. Dhabhar FS, Mcewen BS. Enhancing versus suppressive effects of stress hormones on skin immune function. *Proc Natl Acad Sci USA.* (1999) 96:1059–64. doi: 10.1073/pnas.96.3.1059
41. Huijbers MG, Marx A, Plomp JJ, Le Panse R, Phillips WD. Advances in the understanding of disease mechanisms of autoimmune neuromuscular junction disorders. *Lancet Neurol.* (2022) 21:163–75. doi: 10.1016/S1474-4422(21)00357-4
42. Barnes PJ, Adcock IM. Glucocorticoid resistance in inflammatory diseases. *Lancet.* (2009) 373:1905–17. doi: 10.1016/S0140-6736(09)60326-3
43. Van Staa TP, Leufkens HG, Abenhaim L, Begaud B, Zhang B, Cooper C. Use of oral corticosteroids in the United Kingdom. *QJM.* (2000) 93:105–11. doi: 10.1093/qjmed/93.2.105
44. Fichtner ML, Jiang R, Bourke A, Nowak RJ, O'connor KC. Autoimmune pathology in myasthenia gravis disease subtypes is governed by divergent mechanisms of immunopathology. *Front Immunol.* (2020) 11:776. doi: 10.3389/fimmu.2020.00776
45. Hehir MK, Hobson-Webb LD, Benatar M, Barnett C, Silvestri NJ, Howard JF, Jr., et al. Rituximab as treatment for anti-MuSK myasthenia gravis: multicenter blinded prospective review. *Neurology.* (2017) 89:1069–77. doi: 10.1212/WNL.0000000000004341
46. Nowak RJ, Coffey CS, Goldstein JM, Dimachkie MM, Benatar M, Kissel JT, et al. Phase 2 trial of rituximab in acetylcholine receptor antibody-positive generalized myasthenia gravis: the BeatMG study. *Neurology.* (2021) 98:e376–89. doi: 10.1212/WNL.0000000000013121
47. Rozsa C, Lovas G, Fornadi L, Szabo G, Komoly S. Safety of long-term combined immunosuppressive treatment in myasthenia gravis–analysis of adverse effects of 163 patients. *Eur J Neurol.* (2006) 13:947–52. doi: 10.1111/j.1468-1331.2006.01382.x
48. Manson SC, Brown RE, Cerulli A, Vidaurre CF. The cumulative burden of oral corticosteroid side effects and the economic implications of steroid use. *Respir Med.* (2009) 103:975–94. doi: 10.1016/j.rmed.2009.01.003
49. Bruce BB, Kupersmith MJ. Safety of prednisone for ocular myasthenia gravis. *J Neuroophthalmol.* (2012) 32:212–5. doi: 10.1097/WNO.0b013e3182536558
50. Huscher D, Thiele K, Gromnica-Ihle E, Hein G, Demary W, Dreher R, et al. Dose-related patterns of glucocorticoid-induced side effects. *Ann Rheum Dis.* (2009) 68:1119–24. doi: 10.1136/ard.2008.092163
51. Schacke H, Schottelius A, Docke WD, Strehlke P, Jaroch S, Schmees N, et al. Dissociation of transactivation from transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. *Proc Natl Acad Sci USA.* (2004) 101:227–32. doi: 10.1073/pnas.0300372101
52. Baudy AR, Reeves EK, Damsker JM, Heier C, Garvin LM, Dillingham BC, et al. Delta-9,11 modification of glucocorticoids dissociates nuclear factor-kappaB inhibitory efficacy from glucocorticoid response element-associated side effects. *J Pharmacol Exp Ther.* (2012) 343:225–32. doi: 10.1124/jpet.112.194340
53. Hoffman EP, Schwartz BD, Mengle-Gaw LJ, Smith EC, Castro D, Mah JK, et al. Vamorolone trial in Duchenne muscular dystrophy shows dose-related improvement of muscle function. *Neurology.* (2019) 93:e1312–23. doi: 10.1212/WNL.0000000000008168
54. Mavroudis PD, Van Den Anker J, Conklin LS, Damsker JM, Hoffman EP, Nagaraju K, et al. Population pharmacokinetics of vamorolone (VBP15) in healthy men and boys with duchenne muscular dystrophy. *J Clin Pharmacol.* (2019) 59:979–88. doi: 10.1002/jcph.1388
55. Schijvens AM, Ter Heine R, De Wildt SN, Schreuder MF. Pharmacology and pharmacogenetics of prednisone and prednisolone in patients with nephrotic syndrome. *Pediatr Nephrol.* (2019) 34:389–403. doi: 10.1007/s00467-018-3929-z
56. Frey BM, Frey FJ. Clinical pharmacokinetics of prednisone and prednisolone. *Clin Pharmacokinet.* (1990) 19:126–46. doi: 10.2165/00003088-199019020-00003
57. Matoulkova P, Pavek P, Maly J, Vlcek J. Cytochrome P450 enzyme regulation by glucocorticoids and consequences in terms of drug interaction. *Expert Opin Drug Metab Toxicol.* (2014) 10:425–35. doi: 10.1517/17425255.2014.878703
58. Zhou C, Tabb MM, Nelson EL, Grun F, Verma S, Sadatrafiei A, et al. Mutual repression between steroid and xenobiotic receptor and NF-kappaB signaling pathways links xenobiotic metabolism and inflammation. *J Clin Invest.* (2006) 116:2280–9. doi: 10.1172/JCI26283
59. Chen HL, Li LR. Glucocorticoid receptor gene polymorphisms and glucocorticoid resistance in inflammatory bowel disease: a meta-analysis. *Dig Dis Sci.* (2012) 57:3065–75. doi: 10.1007/s10620-012-2293-2
60. Zou YF, Xu JH, Wang F, Liu S, Tao JH, Cai J, et al. Association study of glucocorticoid receptor genetic polymorphisms with efficacy of glucocorticoids in systemic lupus erythematosus: a prospective cohort study. *Autoimmunity.* (2013) 46:531–6. doi: 10.3109/08916934.2013.830714
61. Quax RA, Koper JW, Huisman AM, Weel A, Hazes JM, Lamberts SW, et al. Polymorphisms in the glucocorticoid receptor gene and in the glucocorticoid-induced transcript 1 gene are associated with disease activity and response to glucocorticoid bridging therapy in rheumatoid arthritis. *Rheumatol Int.* (2015) 35:1325–33. doi: 10.1007/s00296-015-3235-z
62. Xie Y, Meng Y, Li HF, Hong Y, Sun L, Zhu X, et al. GR gene polymorphism is associated with inter-subject variability in response to glucocorticoids in patients with myasthenia gravis. *Eur J Neurol.* (2016) 23:1372–9. doi: 10.1111/ene.13040
63. Xie Y, Li HF, Sun L, Kusner LL, Wang S, Meng Y, et al. The role of osteopontin and its gene on glucocorticoid response in myasthenia gravis. *Front Neurol.* (2017) 8:230. doi: 10.3389/fneur.2017.00230
64. Hamid QA, Wenzel SE, Hauk PJ, Tsicopoulos A, Wallaert B, Lafitte JJ, et al. Increased glucocorticoid receptor beta in airway cells of glucocorticoid-insensitive asthma. *Am J Respir Crit Care Med.* (1999) 159:1600–4. doi: 10.1164/ajrccm.159.5.9804131
65. Derijk RH, Schaaf MJ, Turner G, Datson NA, Vreugdenhil E, Cidlowski J, et al. A human glucocorticoid receptor gene variant that increases the stability of the glucocorticoid receptor beta-isoform mRNA is associated with rheumatoid arthritis. *J Rheumatol.* (2001) 28:2383–8.
66. Piotrowski P, Burzynski M, Lianeri M, Mostowska M, Wudarski M, Chwalinska-Sadowska H, et al. Glucocorticoid receptor beta splice variant expression in patients with high and low activity of systemic lupus erythematosus. *Folia Histochem Cytobiol.* (2007) 45:339–42.
67. Lucafo M, Granata S, Bonten EJ, Mccorkle R, Stocco G, Caletti C, et al. Hypomethylation of NLRP3 gene promoter discriminates glucocorticoid-resistant from glucocorticoid-sensitive idiopathic nephrotic syndrome patients. *Clin Transl Sci.* (2021) 14:964–75. doi: 10.1111/cts.12961
68. Yamanishi Y, Boyle DL, Green DR, Keystone EC, Connor A, Zollman S, et al. p53 tumor suppressor gene mutations in fibroblast-like synoviocytes from erosion synovium and non-erosion synovium in rheumatoid arthritis. *Arthritis Res Ther.* (2005) 7:R12–18. doi: 10.1186/ar1448
69. Spies LL, Verhoog NJD, Louw A. Acquired glucocorticoid resistance due to homologous glucocorticoid receptor downregulation: a modern look at an age-old problem. *Cells.* (2021) 10:2529. doi: 10.3390/cells10102529
70. Maranville JC, Baxter SS, Torres JM, Di Rienzo A. Inter-ethnic differences in lymphocyte sensitivity to glucocorticoids reflect variation in transcriptional response. *Pharmacogenomics J.* (2013) 13:121–9. doi: 10.1038/tpj.2011.55
71. Maranville JC, Micic D, Hanauer SB, Di Rienzo A, Kupfer SS. *In vitro* sensitivity assays and clinical response to glucocorticoids in patients with inflammatory bowel disease. *J Crohns Colitis.* (2014) 8:1539–47. doi: 10.1016/j.crohns.2014.06.013
72. Federico MJ, Covar RA, Brown EE, Leung DY, Spahn JD. Racial differences in T-lymphocyte response to glucocorticoids. *Chest.* (2005) 127:571–8. doi: 10.1378/chest.127.2.571
73. Corwin E, Dunlop AL, Fernandes J, Li S, Pearce B, Jones DP. Metabolites and metabolic pathways associated with glucocorticoid resistance in pregnant African-American women. *Compr Psychoneuroendocrinol.* (2020) 1–2:100001. doi: 10.1016/j.cpnec.2020.100001
74. Maranville JC, Baxter SS, Witonsky DB, Chase MA, Di Rienzo A. Genetic mapping with multiple levels of phenotypic information reveals determinants of lymphocyte glucocorticoid sensitivity. *Am J Hum Genet.* (2013) 93:735–43. doi: 10.1016/j.ajhg.2013.08.005

75. Whitacre CC. Sex differences in autoimmune disease. *Nat Immunol.* (2001) 2:777–80. doi: 10.1038/ni0901-777
76. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol.* (2016) 16:626–38. doi: 10.1038/nri.2016.90
77. Kinder JM, Stelzer IA, Arck PC, Way SS. Immunological implications of pregnancy-induced microchimerism. *Nat Rev Immunol.* (2017) 17:483–94. doi: 10.1038/nri.2017.38
78. Libert C, Dejager L, Pinheiro I. The X chromosome in immune functions: when a chromosome makes the difference. *Nat Rev Immunol.* (2010) 10:594–604. doi: 10.1038/nri2815
79. Ngo ST, Steyn FJ, Mccombe PA. Gender differences in autoimmune disease. *Front Neuroendocrinol.* (2014) 35:347–69. doi: 10.1016/j.yfrne.2014.04.004
80. Loda A, Collombet S, Heard E. Gene regulation in time and space during X-chromosome inactivation. *Nat Rev Mol Cell Biol.* (2022) 23:231–49. doi: 10.1038/s41580-021-00438-7
81. Lucafo M, Bramuzzo M, Selvestrel D, Da Lozzo P, Decorti G, Stocco G. Gender may influence the immunosuppressive actions of prednisone in young patients with inflammatory bowel disease. *Front Immunol.* (2021) 12:673068. doi: 10.3389/fimmu.2021.673068
82. Lee I, Kaminski HJ, Xin H, Cutter G. Gender and quality of life in myasthenia gravis patients from the myasthenia gravis foundation of America registry. *Muscle Nerve.* (2018) 50:90–98. doi: 10.1007/978-3-319-73585-6_11
83. Lee I, Kaminski HJ, Mcpherson T, Feese M, Cutter G. Gender differences in prednisone adverse effects: survey result from the MG registry. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e507. doi: 10.1212/NXI.0000000000000507
84. Duma D, Collins JB, Chou JW, Cidlowski JA. Sexually dimorphic actions of glucocorticoids provide a link to inflammatory diseases with gender differences in prevalence. *Sci Signal.* (2010) 3:ra74. doi: 10.1126/scisignal.2001077

Conflict of Interest: HK is a consultant for Roche, Cabeletta Bio, Lincoln Therapeutics, Takeda and UCB Pharmaceuticals; and is CEO and CMO of ARC Biotechnology, LLC based on US Patent 8,961,98. He is principal investigator of the Rare Disease Network for Myasthenia Gravis (MGNet) National Institute of Neurological Disorders and Stroke, U54 NS115054 and Targeted Therapy for Myasthenia Gravis. R41 NS110331-01 to ARC Biotechnology.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 09 May 2022
doi: 10.3389/fneur.2022.873599

Clinical Characteristics and Prognosis of Anti-AChR Positive Myasthenia Gravis Combined With Anti-LRP4 or Anti-Titin Antibody

Yuping Chen[1], *Xiaoyong Tao*[1], *Yan Wang*[1], *Shengjie Xu*[1], *Yanhua Yang*[1], *Jinming Han*[2]* *and Feng Qiu*[1]*

[1] *Senior Department of Neurology, The First Medical Center of PLA General Hospital, Beijing, China,* [2] *Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China*

OPEN ACCESS

Edited by:
Xiangjun Chen,
Fudan University, China

Reviewed by:
Hongjun Hao,
Peking University First Hospital, China
Bitao Bu,
Huazhong University of Science and Technology, China
Hongyu Zhou,
Sichuan University, China

***Correspondence:**
Feng Qiu
qiufengnet@hotmail.com
Jinming Han
hanjinming1202@126.com

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *11 February 2022*
Accepted: *06 April 2022*
Published: *09 May 2022*

Citation:
Chen Y, Tao X, Wang Y, Xu S, Yang Y, Han J and Qiu F (2022) Clinical Characteristics and Prognosis of Anti-AChR Positive Myasthenia Gravis Combined With Anti-LRP4 or Anti-Titin Antibody. Front. Neurol. 13:873599. doi: 10.3389/fneur.2022.873599

Objective: This study aimed to summarize the clinical characteristics and prognosis of patients with anti- acetylcholine receptor (AChR) positive myasthenia gravis (MG) with a combination of anti-LRP4 or Titin antibodies.

Methods: A total of 188 patients with generalized MG before immunotherapy were retrospectively collected and then divided into three groups: single anti-AChR positive-MG (AChR-MG, 101 cases), anti-AChR combined with anti-low-density lipoprotein receptor-related protein four-positive MG (AChR+LRP4-MG, 29 cases), and anti-AChR combined with anti-Titin-positive MG (AChR+Titin-MG, 58 cases). Clinical manifestations, therapeutic responses to immunotherapy, and follow-up information were analyzed.

Results: Of the 188 seropositive MG patients, 29 (15.4%) were positive for both AChR and LRP4 antibodies, and 58 (30.9%) were positive for both AChR and Titin antibodies. The mean disease onset ages in the three groups were 47.41 ± 7.0, 49.81 ± 9.2, and 48.11 ± 6.5 years, respectively. AChR+LRP4-MG showed female predominance (27.6% were males and 72.4% were females), with mild overall clinical symptoms. The AChR+Titin-MG group showed shorter times for conversion to generalized MG (5.14 ± 0.0 months) than the AChR-MG group (11.69 ± 0.0 months) and the AChR+LRP4-MG group (13.08 ± 0.5 months; P <0.001 in both cases). Furthermore, AChR+Titin-MG group had increased bulbar dysfunction, higher incidences of thymoma (32.8 vs. 19.8% and 3.4%, P=0.035), more severe quantitative MG scores, as assessed by both QMG scores [15.5 (11.75–22.5) vs. 13 (8–19), $P = 0.005$; and 9 (6–14) $P < 0.001$], and MG-ADL scores [10 (8–13) vs. 8 (5–13), $P = 0.018$; and 6 (4–8), $P < 0.001$]. Treatment for AChR+Titin-MG was largely dependent on corticosteroids and immunosuppressive agents (56.7 vs. 19.2% and 16.7%, $p = 0.028$). The rates of achieving s(MMS) or better within 2 years following immunotherapy in the three groups were 51.5, 62.1, and 51.7%, respectively ($P = 0.581$).

Conclusion: Clinical symptoms of anti-AChR positive MG combined with Titin antibody were more severe and progressed faster than those in the AChR + LRP4 and AChR

groups. Regardless of antibody status, all patients responded well to immunotherapy and had relatively good prognoses.

Keywords: acetylcholine receptor, Myasthenia Gravis, low-density lipoprotein receptor-related protein 4, Titin antibody, minimal manifestations status (MMS)

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease involving antibody-mediated destruction of the neuromuscular junction, which causes fatigable weakness (1). There are three confirmed pathogenic antibodies in MG: acetylcholine receptor antibody (AChR-Ab), muscle-specific tyrosine kinase antibody (MuSK-Ab), and low-density lipoprotein receptor-related protein 4 antibody (LRP4-Ab) (1–3). These main concomitant antibodies target different muscle proteins, including titin, myosin, tropomyosin, and the ryanodine receptor (RyR) (3–6). It is generally believed that pathogenic antibodies are closely related to the occurrence, development, and prognosis of autoimmune diseases (7–10). Although the pathogenicity of these concomitant antibodies is unclear, diagnostic and prognostic values for Titin and RyR antibodies have already been established based on intracellular localization of their target antigens (4, 11, 12). Serum antibody detection also plays an important role in the clinical diagnosis of MG, and more than one autoantibody against extracellular or intracellular targets has been noted in patients with MG (13, 14). However, the clinical value of these antibodies remains unclear. In this study, we retrospectively analyzed the prevalence, clinical features, and prognosis of anti-AChR positive MG combined with anti-LRP4 or anti-Titin antibodies.

METHODS

Patient Information

Medical records and follow up data from 1,109 MG patients who were treated in our hospital between January 2013 and December 2019 were retrospectively reviewed and analyzed. The inclusion criteria included: (1) Patients who had been diagnosed with MG and were over 18 years of age. The MG diagnosis was based on fluctuating weakness symptoms along with supporting pharmacological, serologic, and electrophysiologic tests; (2) Onset symptoms and signs were compatible with generalized MG; (3) Patients were not treated with steroids, immunosuppressive agents, IVIG, or plasma exchange for at least 6 months before antibody detection; (4) Anti-AChR, MuSK, LRP4, and Titin-Ab were measured; (5) Patients were seropositive for anti-AChR antibody.

The following patients were excluded from the study: (1) A total of 171 patients who were younger than 18 years old at the time of admission; (2) Patients who had ocular MG or who only had ocular muscle involvement but for <2 years (180 cases); (3) Patients who had been treated with immunosuppressive agents (tacrolimus in 62 cases, cyclosporine in 78 cases, azathioprine in 20 cases, cyclophosphamide in 40 cases and steroids in 280 cases), had plasma exchanges (PLEX) or had intravenous immunoglobulin (IVIG) treatment (30 cases) within 6 months prior to antibody detection; (4) Patients who were negative for anti-AChR (32 cases); (5) Patients who had incomplete data regarding anti-AChR; or within whom Musk, LRP4, and Titin-Ab were not detected (28 cases). Pregnant individuals were also excluded in this study. We ultimately enrolled 188 patients in our study (**Figure 1**).

Myasthenia gravis was diagnosed by senior neurologists based on the guidelines of the International Consensus Guidance for Management of Myasthenia Gravis (2, 5). Patients were classified into three groups: AChR-MG, AChR+LRP4-MG, and AChR+Titin-MG. Clinical, diagnostic, therapeutic, and prognosis data, including gender, age of onset, initial symptoms, disease progression, clinical classification, disease severity, the incidence of myasthenia crisis, thymus histopathology, therapeutic options, and prognosis were collected.

Antibody Testing

All patients were tested for MG-related antibodies in the serum before immunotherapy. If AChR Ab was positive, MuSK, LRP4, Titin, and RyR Ab were further tested in these patients.

A radioimmunoassay for the AChR antibody was performed according to the manufacturer's protocol (RSR Limited, United Kingdom). Patients were defined as antibody positive if antibody titers were ≥0.5 nmol/l (AChR Ab). Blood was tested for MuSK, LRP4, and Titin using enzyme-linked immunoassay (ELISA) as previously described (15). The investigators who performed the ELISA experiments were blinded to clinical diagnoses.

Therapy

Therapeutic strategies for generalized MG include acetylcholinesterase inhibitors and immunotherapeutic agents. Symptomatic treatment with oral pyridostigmine bromide was used in patients who responded positively to the neostigmine trial. Most patients with generalized MG require induction therapy with glucocorticosteroids. During therapeutic periods, the steroid dosage was gradually increased or decreased according to patients' conditions. Immunosuppressive agents, including azathioprine, cyclosporine A, tacrolimus, or cyclophosphamide, were used in combination with corticosteroids if needed. If the disease was severe (i.e., involved respiratory muscle and bulbar muscle), patients were treated with IVIG and plasma exchanges. These patients were followed up for 2 years. Patients who received either azathioprine, tacrolimus, cyclosporine, or cyclophosphamide were considered to receive immunosuppressive therapy.

Prognosis

Clinical status and disease severity were evaluated based on MGFA classifications, quantitative MG scores (QMGs), and the

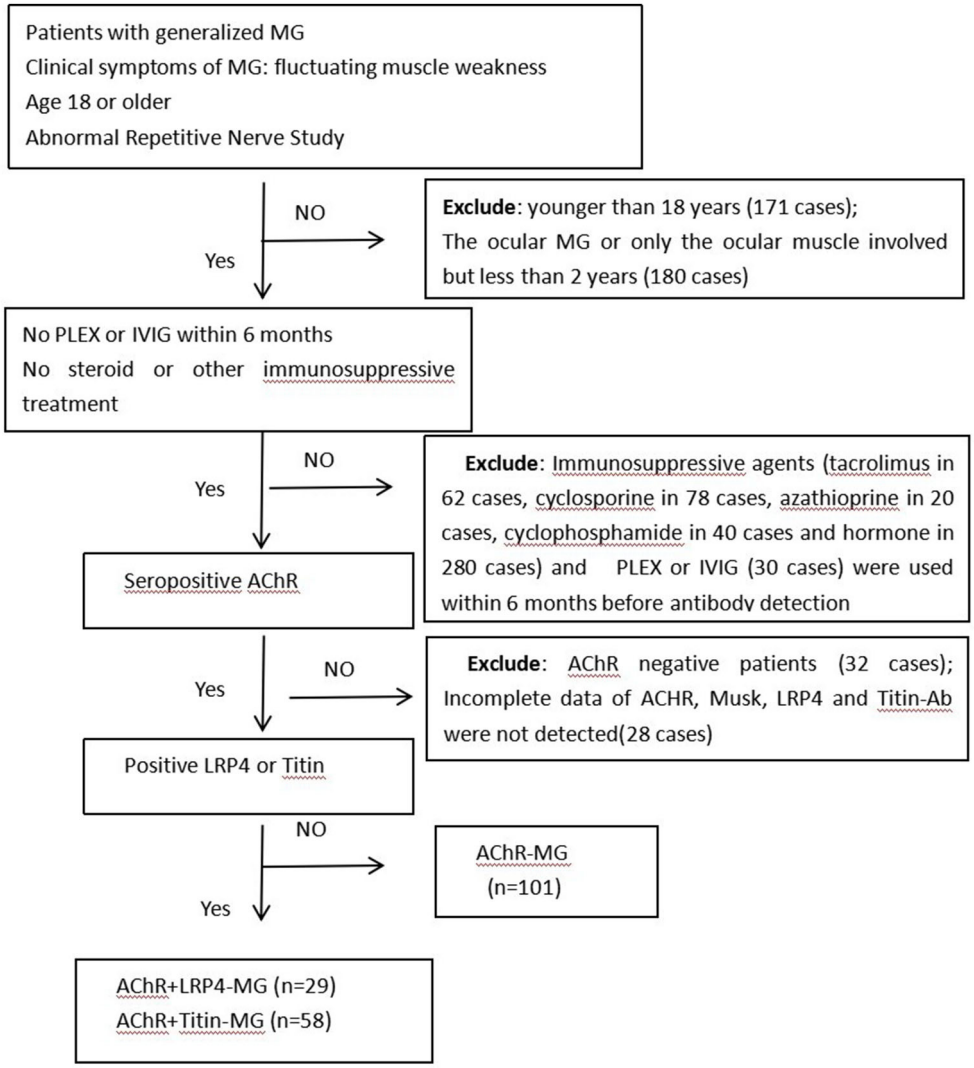


FIGURE 1 | Flowchart of participants included in this study. Abbreviations: MG, Myasthenia gravis; PLEX, plasma exchange; IVIG, intravenous immunoglobulin; AChR, acetylcholine receptor; LRP4, low-density lipoprotein receptor-related protein 4; n, number of patients.

daily living scale (MG-ADL), respectively. In terms of MGFA post-intervention status (PIS), the classification of "Minimal Manifestation Status (MMS) or better" included minimal manifestation Status (MM0-3), pharmacological remission (PR), and complete stable remission (CSR).

All individuals were followed up and evaluated for 2 years after different treatments. The study was stopped after 2 years. The proportion of patients in the three groups who reached an "MMS or better" state after treatment, and maintained it for more than 6 months were analyzed.

Statistical Analysis

SPSS 26.0 statistical software (IBM, Armonk, New York) was used for statistical analysis. Categorical data were represented as frequencies (%). Continuous data were represented as mean±standard deviation (SD), and ANOVA tests were used for quantitative data. The median (interquartile interval) was used for non-normally distributed statistical descriptions, and nonparametric tests were used for inter-group comparisons. Qualitative statistics were evaluated using two-tailed Fisher's exact tests. A $P < 0.05$ was considered statistically significant.

RESULTS

Demographic Information

Of the 188 seropositive generalized MG patients, 29 patients were positive for both AChR and LRP4 antibodies, while 58 cases were positive for both AChR and Titin. The mean age of disease onset was 47.41 ± 7.0, 49.81 ± 9.2, and 48.11 ± 6.5 years in the AChR-MG, AChR+LRP4-MG, and AChR+Titin-MG groups, respectively. AChR+LRP4-MG showed female predominance (27.6 vs. 72.4%). The proportion of men and women in the

TABLE 1 | Characteristics of patients with AChR-MG, AChR+LRP4-MG and AChR+Titin-MG.

	AChR MG ($n = 101$)	AChR+LRP4 MG ($n = 29$)	AChR+Titin MG ($n = 58$)	*P*-value (AchR+LRP4 MG vs. AchR MG)	*P*-value (AchR+Titin MG vs. AchR MG)
Sex					
Men	49 (48.5%)	8 (27.6%)	34 (58.6)	0.045	0.219
Women	52 (51.5%)	21 (72.4%)	24 (41.4%)		
Onset age (years)	47.41 ± 7.0	49.81 ± 9.2	48.11 ± 6.5	0.498	0.809
Onset distribution					
Ocular	86 (85.1%)	20 (69.0%)	49 (84.5%)	0.000	0.481
Bulbar	8 (7.9%)	1 (3.4%)	7 (12.1%)		
Limb	7 (6.9%)	8 (27.6%)	2 (3.4%)		
Time from ocular onset to other muscle (months)	11.69 ± 0.0	13.08 ± 0.5	5.14 ± 0.0	0.472	0.000
Myasthenic crisis	18(17.8%)	2(6.9%)	15(25.9%)		
Thymoma	20(19.8%)	1(3.4%)	19(32.8%)	0.068	0.035
MGFA					
IIa	25 (24.8%)	14 (48.3%)	7 (12.1%)	0.02	
IIIa	4 (4.0%)	1 (3.4%)	4 (6.9%)		
IVa	2 (2.0%)	0 (0)	0 (0)		
IIb	15 (14.9%)	5 (17.2%)	4 (6.9%)		
IIIb	21 (20.8%)	2 (6.9%)	15 (25.9%)		
IVb	16 (15.8%)	5 (17.2%)	14 (24.1%)		
V	18 (17.8%)	2 (6.9%)	14 (24.1%)		
QMG scores	13 (8–19)	9 (6–14)	15.5 (11.8–22.5)	0.008	0.005
MG ADL scores	8 (5–13)	6 (4–8)	10 (8–13)	0.009	0.018
Thymectomy	26 (25.7%)	3 (10.3%)	22 (37.9%)	0.127	0.151

Comparison of clinical data among the three groups was done by ANOVA test or Fisher exact test or nonparametric test. Abbreviations: MG, Myasthenia gravis; AChR, acetylcholine receptor; LRP4, low-density lipoprotein receptor-related protein 4; MGAF, Myasthenia Gravis Foundation of America; QMG, quantitative MG score; MG-ADL, MG-specific activities of daily living scale. The data are shown as mean±SD or ratio or median (interquartile interval).

AChR-MG and AChR+Titin-MG groups was relatively equal (48.5 vs. 51.5, 58.6 vs. 41.4%; respectively), and there were no significant gender differences between the two groups (**Table 1**).

Clinical Characteristics

Ocular muscle weakness was the most common onset symptom in all three groups. More patients in the AChR+LRP4-MG suffered from limb weakness onset than in the AChR-MG and AChR+Titin-MG groups (27.6 vs. 6.9% and 11.5%; $P < 0.001$). Compared to the AChR-MG and AChR+LRP4-MG groups, patients in the AChR+Titin-MG group tended to have shorter conversion times from ocular to generalized MG (5.14 ± 0.0 vs. 11.69 ± 0.0 and 13.08 ± 0.5 months; $P < 0.001$), Furthermore, AChR+Titin-MG patients had greater bulbar dysfunction, higher incidences of thymoma (32.8 vs. 19.8 and 3.4%; $P = 0.006$), and more severe QMG scores [15.5 (11.75–22.5) vs. 13 (8–19) in AChR-the MG group (P=0.005), and 9 (6–14) in the AChR+LRP4-MG group ($P < 0.001$)]. MG-ADL scores were also significantly increased in the AChR+Titin-MG group [10 (8–13) vs. 8 (5–13) in the AChR-MG group, $P = 0.018$; and 6 (4–8) in the AChR+LRP4-MG group, $P < 0.001$].

The most common MGFA classification in AChR+LRP4-MG patients was MGFA IIa (48.3%), while 25.9% of AChR+Titin-MG patients were classified as MGFA IIIb. Additionally, more patients were classified as MGFA IVb-V in the AChR+Titin-MG group than in either the AChR-MG and AChR+LRP4-MG groups (48.2 vs. 33.2, and 24.1%, $P = 0.02$).

Affected muscles in the three groups were analyzed at different time points (6 months, 12 months, and 24 months; see **Figure 2**). Our results showed that clinical symptoms did not differ significantly among the three groups during different time points.

Treatment and Prognosis

Patients were treated with standard therapies for MG. The rates of achieving MMS or better in the three groups within 2 years after immunosuppressive treatment were 51.5, 62.1, and 51.7%, respectively (**Table 2**). AChR+Titin-MG treatment was highly dependent on steroids combined with immunosuppressive agents (**Table 3**).

DISCUSSION

Our study found that clinical symptoms of anti-AChR MG combined with anti-LRP4 or anti-Titin antibody were more severe and progressed faster than anti-AChR positive MG. Regardless of antibody status, all patients responded well to immunotherapy and had relatively good prognoses.

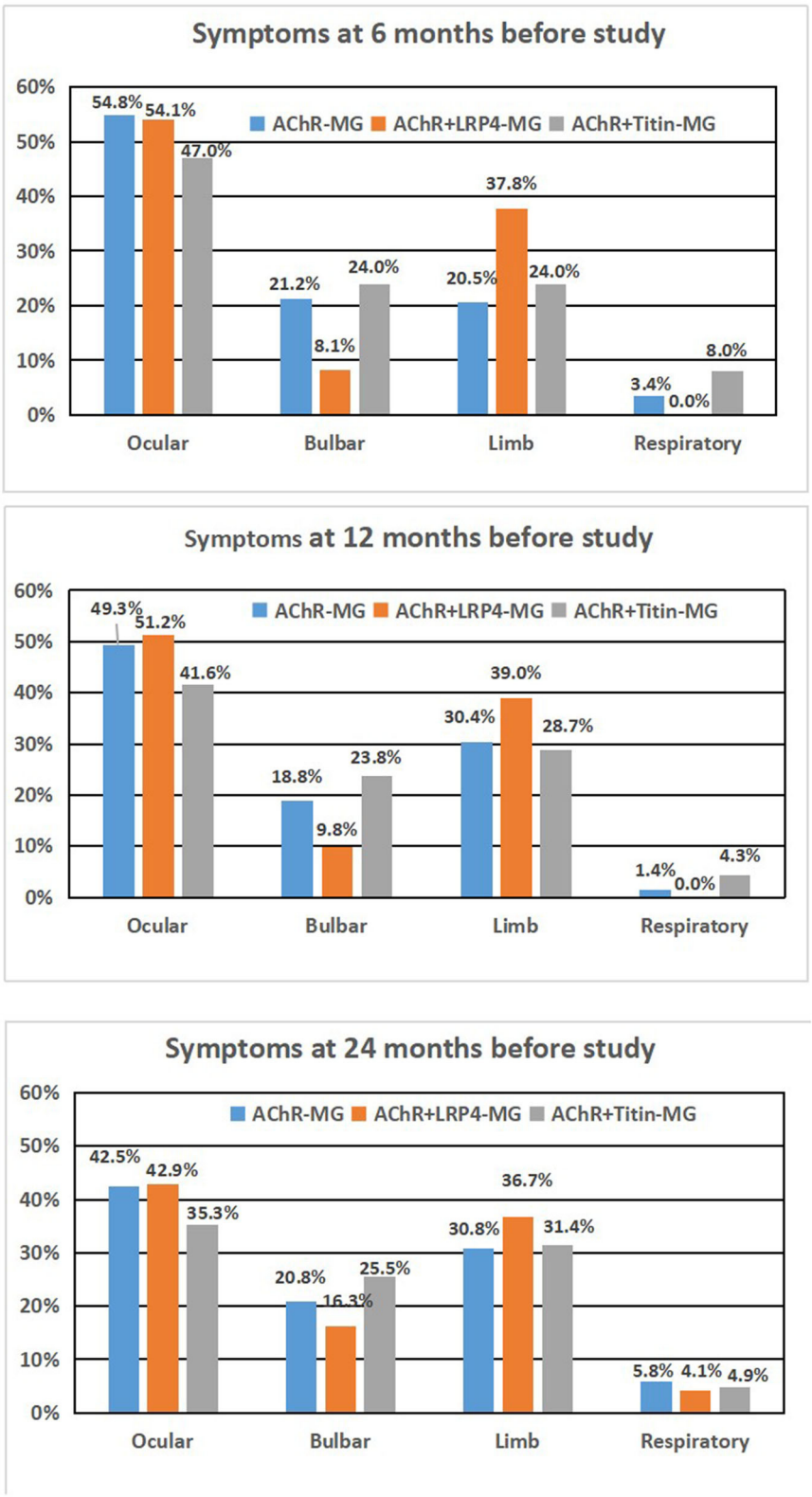


FIGURE 2 | Syptoms of AChR-MG, AChR+LRP4-MG, and AChR+Titan-MG patients during different time period. **(A)** 6 months before study, **(B)** 12 months before study, and **(C)** 24 months before study.

TABLE 2 | Comparison of the prognosis after immunosuppressive therapy among AchR-MG, AchR+LRP4 and AchR+Titin MG.

	AchR-MG	AchR+LRP4	AchR+Titin	*P*-value
MM	52/101 (51.5%)	18/29 (62.1)	30/58 (51.7%)	0.581
MM-0 or better	9/101 (8.9%)	2/29 (6.9%)	2/58 (3.4%)	0.454
MM-1	10/101 (9.9%)	5/29 (17.2%)	4/58 (6.9%)	0.358
MM-2	15/101 (14.9%)	6/29 (20.7%)	8/58 (13.8%)	0.799
MM-3	18/101 (17.8%)	5/29 (17.2%)	16/58 (27.6%)	0.31

Minimal Manifestations (MM), The patient has no symptoms of functional limitations from MG but has some weakness on examination of some muscles. This class recognizes that some patients who otherwise meet the definition of CSR or PR do have weakness that is only detectable by careful examination; MM-0, The patient has received no MG treatment for at least 1 year; MM-1, The patient continues to receive some form of immunosuppression but no cholinesterase inhibitors or other symptomatic therapy; MM-2, The patient has received only low-dose cholinesterase inhibitors (<120 mg pyridostigmine/day) for at least 1 year; MM-3, The patient has received cholinesterase inhibitors or other symptomatic therapy and some form of immunosuppression during the past year.

TABLE 3 | Therapeutic strategy for MMS or better among three groups.

	AchR MG	AchR+LRP4 MG	AchR+Titin MG	*P*-value
steroid	14 (26.9%)	5 (27.8%)	6 (20.0%)	0.819
Immunosuppressant	28 (53.9%)	10 (55.6%)	7 (23.3%)	0.017
steroid+ Immunosuppressant	10 (19.2%)	3 (16.7%)	17 (56.7%)	0.001

Demographic Characteristics

About 85% of MG patients have autoantibodies against AChR, whereas 5%-26% of MG have autoantibodies against MuSK (1–3, 15–17). We focused on AChR positive patients in this study. We only found one patient who was both AChR and MuSK antibody-positive, which was lower than the proportion of patients who had seronegative MG (and who were not excluded in our study).

Most previous studies on LRP4 have largely focused on the seronegative MG population. However, there have been some reports of anti-AChR patients with double-positive LRP4 antibodies in clinical practice (20). Among 1,109 patients diagnosed with MG at our center, we found 29 cases with AChR combined with anti-LRP4 antibodies. The proportion of patients with anti-LRP4-antibodies was 2.61%, which coincided with the proportion in double-negative patients (14) and in other studies (18–20). Titin auto-antibodies were found in 30.9% of seropositive patients [compared with the 20–40% that has previously been reported in the literature (11, 13, 21). We found that the AChR+LRP4-MG phenotype showed a strong female predominance (72.4%), which was consistent with previous studies (22, 23). The mean age of onset in the three groups was 47.41 ± 7.0, 49.81 ± 9.2, and 48.11 ± 6.5 years, respectively. We focused on adults with generalized MG and excluded relatively young ocular patients.

Clinical Features

Most MG patients with ocular symptoms at onset may progress to the generalized form of the disease within 2 years (1, 3). Our results confirmed that most MG patients had an ocular-only onset. However, the AChR+LRP4-MG group had significantly higher numbers of patients with limb weakness during disease onset than the AChR-MG or AChR+Titin-MG group. Therefore, AChR+LRP4-MG patients were much more likely to have generalized MG at the time of disease onset. Three of the AChR+LRP4-MG patients presented with MGFA class V in our study (20). It is unknown if other antibodies, such as agrin, were positive because that testing was not done (14).

Compared to the AChR-MG and AChR+LRP4-MG groups, AChR+Titin-MG patients showed shorter progression times from ocular to generalized MG (within 5.1 months). Rapid disease progression following symptom onset maybe because of the involvement of titin antibodies. Additionally, our data on MGFA classifications showed that 25.9% of AChR+Titin-MG patients were classified as MGFA IIIb, while 48.3% of AChR+LRP4-MG patients were classified as MGFA IIa. Moreover, there were more patients with MGFA IVb -V. Our results indicated that AChR+Titin-MG was associated with more severe disease status. Titin antibodies are usually considered to be accompanying antibodies and can only be found in patients with MG and anti-AChR antibodies. It is highly likely that the presence of thymoma in AChR+Titin-MG patients is related to their disease pathology.

Muscles that were involved at different time points (i.e., 6, 12, and 24 months before our study) did not differ significantly among the three groups. Thus, affected muscle groups appear to be similar at different stages of the disease, although disease severity differs.

Treatment and Prognosis

Current common treatments for MG include AChE inhibitors, immunosuppressive drugs, thymectomy, IVIG, and plasmapheresis (24, 25). In our study, the proportions of patients who have achieved MM-3 or better for more than 6 months in the three groups were 51.5, 62.1, and 51.7%, respectively. These percentages are higher than what was reported in a study conducted by Utsugisawa K (26) but are consistent with other previous studies (27, 28).

All patients were treated with pyridostigmine. Monotherapy with an immunosuppressive agent was used in 53.9 and 55.6% of AChR-MG and AChR+LRP4-MG patients, and immunosuppressive therapy was used in combination therapy with azathioprine or tacrolimus corticosteroids in 56.7% of patients with AChR+Titin-MG.

The proportion of steroids combined with immunosuppressive agents in the AChR+Titin MG group was much higher than in the other two groups, suggesting that AChR+Titin MG needs stronger immunotherapy to achieve the same outcomes and is thus also associated with severe immune dysfunction.

In summary, anti-AChR positive MG can coexist with anti-LRP4 or anti-Titin antibodies. AChR+LRP4-MG has a female predominance and presents with milder symptoms. Furthermore, AChR+Titin-MG shows a shorter conversion time from ocular to generalized MG, a higher incidence of thymoma, and has a more severe presentation than AChR+LRP4-MG.

Regardless of antibody status, all patients responded well to immunotherapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The First Medical Center of PLA General Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YC and XT acquired the clinical data, reviewed the literature, and drafted the article. JH and FQ designed the study, supervised the initial drafting, and critically revised the article. YW, SX and YY collected and analyzed the clinical data. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Gilhus NE. Myasthenia gravis. *N Engl J Med.* (2016) 375:2570–81. doi: 10.1056/NEJMra1602678
2. Berrih-Aknin S, Frenkian-Cuvelier M, Eymard B. Diagnostic and clinical classification of autoimmune myasthenia gravis. *J Autoimmun.* (2014) 48-9:143–8. doi: 10.1016/j.jaut.2014.01.003
3. Lazaridis K, Tzartos SJ. Myasthenia Gravis: autoantibody specificities and their role in MG management. *Front Neurol.* (2020) 11:596981. doi: 10.3389/fneur.2020.596981
4. Skeie GO, Aarli JA, Gilhus NE. Titin and ryanodine receptor antibodies in myasthenia gravis. *Acta Neurol Scand Suppl.* (2006) 183:19–23. doi: 10.1111/j.1600-0404.2006.00608.x
5. Dresser L, Wlodarski R, Rezania K, Soliven B. Myasthenia gravis: epidemiology, pathophysiology and clinical manifestations. *J. Clin. Med.* (2021) 10:2235. doi.org/10.3390/jcm10112235. doi: 10.3390/jcm10112235
6. Yamamoto T, Sato T, Sugita H. Antifilamin, antivinculin, and antitropomyosin antibodies in myasthenia gravis. *Neurology.* (1987) 37:1329–33. doi: 10.1212/WNL.37.8.1329
7. Cordts I, Bodart N, Hartmann K, Karagiorgou K, Tzartos JS, Mei L, et al. Screening for lipoprotein receptor-related protein 4-, agrin-, and titin-antibodies and exploring the autoimmune spectrum in myasthenia gravis. *J Neurol.* (2017) 264:1193–203. doi: 10.1007/s00415-017-8514-z
8. Illa I, Cortes-Vicente E, Martinez MA, Gallardo E. Diagnostic utility of cortactin antibodies in myasthenia gravis. *Ann N Y Acad Sci.* (2018) 1412:90–4. doi: 10.1111/nyas.13502
9. Gasperi C, Melms A, Schoser B, Zhang Y, Meltoranta J, Risson V, et al. Anti-agrin autoantibodies in myasthenia gravis. *Neurology.* (2014) 82:1976–83. doi: 10.1212/WNL.0000000000000478
10. Fichtner ML, Jiang R, Bourke A, Nowak RJ. and O'Connor KC. Autoimmune pathology in myasthenia gravis disease subtypes Is governed by divergent mechanisms of immunopathology. *Front Immunol.* (2020) 11:776. doi: 10.3389/fimmu.2020.00776
11. Stergiou C, Lazaridis K, Zouvelou V, Tzartos J, Mantegazza R, Antozzi C, et al. Titin antibodies in "seronegative" myasthenia gravis–a new role for an old antigen. *J Neuroimmunol.* (2016) 292:108–15. doi: 10.1016/j.jneuroim.2016.01.018
12. Romi F, Aarli JA, Gilhus NE. Myasthenia gravis patients with ryanodine receptor antibodies have distinctive clinical features. *Eur J Neurol.* (2007) 14:617–20. doi: 10.1111/j.1468-1331.2007.01785.x
13. Somnier FE, Engel PJ. The occurrence of anti-titin antibodies and thymomas: a population survey of MG 1970-1999. *Neurology.* (2002) 59:92–8. doi: 10.1212/WNL.59.1.92
14. Rivner MH, Quarles BM, Pan JX, Yu Z, Corse A, Dimachkie MM, et al. Clinical features of LRP4/agrin-antibody-positive myasthenia gravis: A multicenter study. *Muscle Nerve.* (2020) 62:333–43. doi: 10.1002/mus.26985
15. Zhang Z, Guan Y, Han J, Li M, Shi M, Deng H. Regional features of MuSK antibody-positive myasthenia gravis in northeast China. *Front Neurol.* (2020) 11:516211. doi: 10.3389/fneur.2020.516211
16. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis-autoantibody characteristics and their implications for therapy. *Nat Rev Neurol.* (2016) 12:259–68 doi: 10.1038/nrneurol.2016.44
17. Ohta K, Shigemoto K, Fujinami A, Maruyama N, Konishi T, Ohta M. Clinical and experimental features of MuSK antibody positive MG in Japan. *Eur J Neurol.* (2007) 14:1029–34. doi: 10.1111/j.1468-1331.2007.01870.x
18. Pevzner A, Schoser B, Peters K, Cosma NC, Karakatsani A, Schalke B, et al. Anti-LRP4 autoantibodies in AChR- and MuSK-antibody-negative myasthenia gravis. *J Neurol.* (2012) 259:427–35. doi: 10.1007/s00415-011-6194-7
19. Li Y, Zhang Y, Cai G, He D, Dai Q. Anti-LRP4 autoantibodies in Chinese patients with myasthenia gravis. *Muscle Nerve.* (2017) 56:938–42. doi: 10.1002/mus.25591
20. Zisimopoulou P, Evangelakou P, Tzartos J, Lazaridis K, Zouvelou V, Mantegazza R, et al. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. *J Autoimmun.* (2014) 52:139–45. doi: 10.1016/j.jaut.2013.12.004
21. Szczudlik P, Szyluk B, Lipowska M, Ryniewicz B, Kubiszewska J, Dutkiewicz M, et al. Antititin antibody in early- and late-onset myasthenia gravis. *Acta Neurol Scand.* (2014) 130:229–33. doi: 10.1111/ane.12271
22. Tsivgoulis G, Dervenoulas G, Kokotis P, Zompola C, Tzartos JS, Tzartos SJ, et al. Double seronegative myasthenia gravis with low density lipoprotein-4 (LRP4) antibodies presenting with isolated ocular symptoms. *J Neurol Sci.* (2014) 346:328–30. doi: 10.1016/j.jns.2014.09.013
23. Higuchi O, Hamuro J, Motomura M, Yamanashi Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. *Ann Neurol.* (2011) 69:418–22. doi: 10.1002/ana.22312
24. Morren J, Li Y. Maintenance immunosuppression in myasthenia gravis, an update. *J Neurol Sci.* (2020) 410:116648. doi: 10.1016/j.jns.2019.116648
25. Lee JI, Jander S. Myasthenia gravis: recent advances in immunopathology and therapy. *Expert Rev Neurother.* (2017) 17:287–99. doi: 10.1080/14737175.2017.1241144
26. Utsugisawa K, Nagane Y, Akaishi T, Suzuki Y, Imai T, Tsuda E, et al. Early fast-acting treatment strategy against generalized myasthenia gravis. *Muscle Nerve.* (2017) 55: 794–801. doi: 10.1002/mus.25397
27. Zhang C, Bu B, Yang H, Wang L. Liu w, Duan RS et al. Immunotherapy choice and maintenance for generalized myasthenia gravis in China. *CNS Neurosci Ther.* (2020) 26:1241–54. doi: 10.1111/cns.13468

28. Fan Z, Li Z, Shen F, Zhang X. Lei L,Su S, et al. Favorable effects of tacrolimus monotherapy on myasthenia gravis patients. *Front Neurol.* (2020) 11:594152. doi: 10.3389/fneur.2020.594152

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 23 May 2022
doi: 10.3389/fneur.2022.880045

Minimal Manifestation Status Indicates a Stable State in Myasthenia Gravis: A Quantitative Study

Ping Jiang[1†], Jie Li[2†], Hong-Yan Li[3], Bin Zhang[3], Yao-Xian Yue[3], Su-Yun Wang[3], Xi-Cun Zi[3], Shuang-Shuang Liu[1], Yi-Fan Li[1], Li-Dong Jiao[1] and Hai-Feng Li[1]*

[1] Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China, [2] Department of Neurology, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [3] Department of Neurology, Qilu Hospital (Qingdao), Cheeloo College of Medicine, Shandong University, Qingdao, China

OPEN ACCESS

Edited by:
Xin-Ming Shen,
Mayo Clinic, United States

Reviewed by:
Kimiaki Utsugisawa,
Hanamaki General Hosipital, Japan
Sarah Hoffmann,
Charité Universitätsmedizin
Berlin, Germany

***Correspondence:**
Hai-Feng Li
drlhf@163.com

[†] These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: 20 February 2022
Accepted: 26 April 2022
Published: 23 May 2022

Citation:
Jiang P, Li J, Li H-Y, Zhang B, Yue Y-X, Wang S-Y, Zi X-C, Liu S-S, Li Y-F, Jiao L-D and Li H-F (2022) Minimal Manifestation Status Indicates a Stable State in Myasthenia Gravis: A Quantitative Study. Front. Neurol. 13:880045. doi: 10.3389/fneur.2022.880045

Introduction: Minimal manifestation (MM) or better was recommended as the treatment goal for myasthenia gravis (MG). The sustainability of this status has not been described quantitatively in patients who had attained or are close to it.

Methods: Patients who were with no or slight impact on daily living were recruited and followed at baseline and 3, 6, and 12 months. The included patients were classified into 3 post-intervention status (PIS) categories: remission (R), MM, and slight impact (SI). The proportion of patients belonging to real-time (not considering the intervals between assessments) and sustained (considering the intervals between assessments) PIS categories was compared at each follow-up. A sensitivity analysis (SA) cohort was established by including patients with PIS categories in all four follow-ups. The QMGS, MG-ADL, and MG-QOL15 scores in patients belonging to each PIS category at each follow-up were compared. The sustainability of the R/MM status was examined and correlated with real-time R/MM status at follow-ups.

Results: At baseline, 376 patients could be classified, including 55 as R (14.2%), 209 as MM (54.0%), and 112 as SI (28.9%). In the whole cohort, 68.8–89.7%, 71–76.7% and 19.8–77.1% of the patients classified into real-time R, MM, and SI categories remained unchanged in each follow-up compared with the previous follow-up. The proportion of patients belonging to each real-time or sustained R/MM status at the three follow-ups was 89.7–92.1 or 60.8–67. In the SA cohort, at least 86.4% of the baseline R/MM patients remained in R/MM status till 12 months. There were no differences in keeping real-time R/MM status at 6 or 12 months between patients with and without sustained R/MM status at 3 and 6 months. There were differences in the QMGS, MG-ADL, and MG-QOL15 scores among patients belonging to each real-time category at baseline and follow-ups, ranking as R<MM<SI. The same trend was observed in patients belonging to each sustained PIS category with smaller scores than the same items of real-time categories.

Conclusion: The sustainability of the R/MM status was confirmed. The R/MM status indicated a stable state of MG. The QMGS, MG-ADL, and MG-QOL15 scores may provide a quantitative reference for these PIS.

Keywords: myasthenia gravis, minimal manifestation status, quantitative myasthenia gravis score, myasthenia gravis activities of daily living, quality of life for myasthenia gravis

INTRODUCTION

In the international consensus guidance for managing myasthenia gravis (MG), minimal manifestation (MM) status or better was recommended as the main component of the treatment goal (1). MM refers to no symptoms or functional limitations from MG but some weakness on examination of some muscles (2). Remission and MM are the mildest end in the post-intervention status (PIS) classification. However, the proportion of patients achieving complete stable remission was only 7–20% and has not improved greatly compared with the 1940s (3). In 2011, Utsugisawa et al. proposed a practical goal of achieving "MM or better" status as the treatment goal (4). The sustainability of this status has not been described quantitatively during the follow-up in patients who had attained or been close to this status yet.

The current definition of MM status relies exclusively on patients' assessments of their symptoms and the impact on their daily living. There are few studies to provide a qualitative reference for the definition of the MM status. The quantitative MG score (QMGS), the MG activities of daily living (MG-ADL), and the 15-item MG quality of life scale (MG-QOL15) are validated measures in the evaluation of MG. One study reported the relevant QMGS and MG-QOL15 in remission or MM status (5). Other similar definitions have been defined recently. In "Patient-acceptable symptom states", the ranges and thresholds of QMGS, MG-ADL, and MG-QOL15 were reported (6). "Minimal symptom expression" was defined as MG-ADL total score of 0–1 or MG-QOL15 total score of 0–3 as the thresholds (7).

In this study, we recruited MG patients who reported no or slight impact on their daily living at baseline, and described the changes and sustainability of the PIS from the baseline through 3, 6, and 12 months after inclusion, and explored the ranges and thresholds of the QMGS, MG-ADL, and MG-QOL15 scores in patients belonging to each PIS classification.

PATIENTS AND METHODS

Included Patients

Patients were recruited consecutively and followed up from March 2017 to May 2019 at the Qilu Hospital of Shandong University (Qingdao). The diagnosis of MG was based on: (1) typical symptoms of fluctuating muscle weakness; (2) positive result of fatigue test; (3) unequivocal positive result of neostigmine test; (4) positive AChR antibody or positive MuSK antibody or amplitude decrement >10% on low-frequency RNS. The included patients should have 1, 2, and 3 as the essential conditions for the diagnosis, and at least one item in 4 as the supporting conditions. The patients were on symptomatic treatment and/or immunosuppressive treatment or were not on any treatments for MG in the setting of the outpatient management. The patients were requested to report their symptoms and the impact of symptoms on their daily living and were included in this study when they were asymptomatic, symptomatic with no impact or slight impact (SI) at the baseline. The patients who reported baseline moderate/severe impact (MSI) on daily living were excluded from this study. The patients with severe anxiety, depression, cognitive impairment, and poor understanding or cooperation in the assessment of QMG, ADL, and QOL were also excluded.

Data on clinical features and treatments of the included patients were collected, including gender, age of onset, current age, disease duration and current clinical classification (ocular or generalized), pyridostigmine bromide (PB) dose, corticosteroid (CS) dose (as prednisone equivalent dose), type and dosage of immunosuppressants (IS), and at each follow-up were recorded. This study was approved by the ethics committee of the hospital, and informed consent has been obtained from all MG patients.

Follow-Up and Data Acquisition

Patients were requested to be followed up at 90 ± 20-day intervals, spontaneous follow-ups were encouraged as needed when their condition changed. At each scheduled follow-up, patients were required to report which impact category they belong to (1) asymptomatic; (2) symptomatic with no impact on daily living, (3) symptomatic with SI on daily living; (4) symptomatic with MSI on daily living. Then, an assessment of MG-ADL and MG-QOL15 was conducted with the assistance of an experienced physician or nurse. Necessary explanations were allowed only when the patients inquired about some confusion in the understanding of the scales. In case of confusion on whether some conditions were caused by MG, the assessment persons helped them to analyze based on their reported symptoms, medical history, and relevant physiological examinations (e.g., dyspnea due to asthma), but avoided replacing their judgment. Meanwhile, the judgment of the accompanying family members should be strictly prohibited to avoid affecting the judgment of patients themselves. The self-report of impact categories, MG-ADL and MG-QOL15 was based on the status of patients most of the time for the last 10 days before each follow-up, with regular PB taking as needed. As we found that almost 2/5 of the patients reported different impact categories when taking PB or not during the same period, the data at the follow-ups with irregular PB taking were not included in the analysis of MG-ADL and MG-QOL15. Subsequently, QMGS was assessed by the principal investigator (Li HF) when the last dose of PB was taken

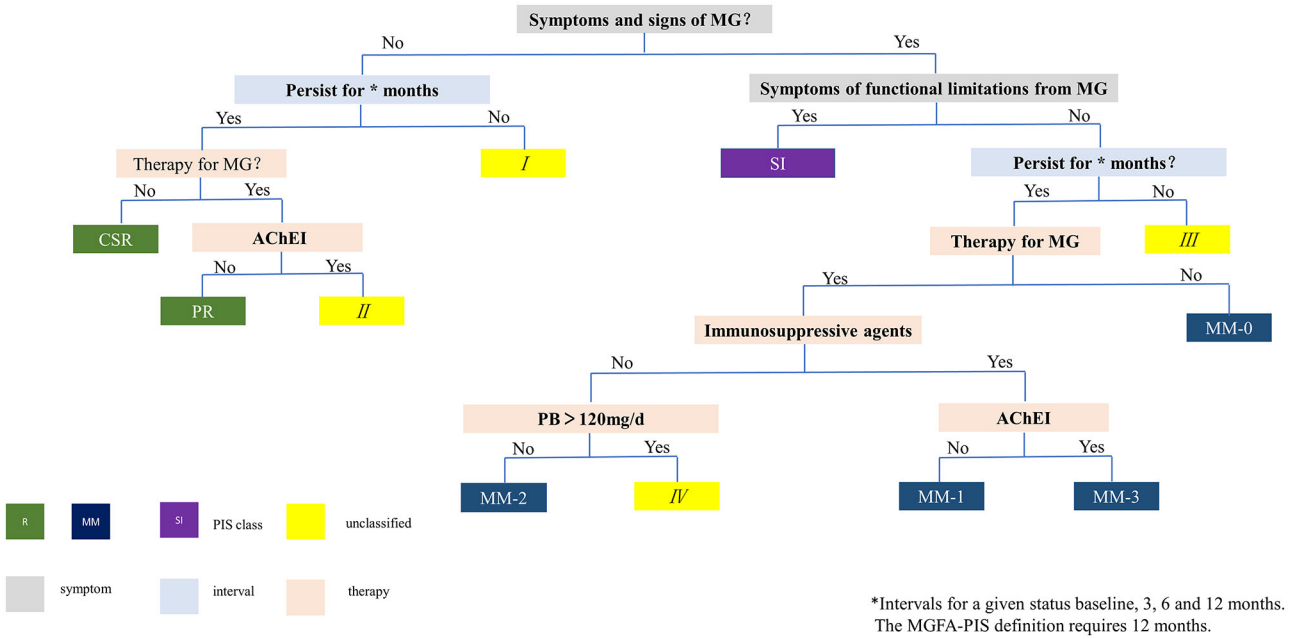


FIGURE 1 | Flowchart for PIS classification. I, III: unable to maintain requested intervals (3, 6 or 12 months), therefore cannot be classified. II: no symptoms, normal fatigue test, but still on oral PB, therefore does not meet the definition of R. IV: PB dose >120 mg/d, therefore does not meet the definition of MM-2. MG, myasthenia gravis; PIS, post-intervention status; AChEI, acetylcholinesterase inhibitor; PB, pyridostigmine bromide; R, remission; CSR, complete stable remission; PR, pharmacological remission; MM, minimal manifestation; SI, slight impact on daily living.

> 6 h before. The patients were asked whether they were too tired or hungry, and were encouraged to cooperate as much as possible during the QMGS assessment to avoid being judged as more severe than their daily condition. The data on the follow-ups with such conditions or poor cooperation due to medical or physiological interference (heart or lung diseases, electrolyte disturbance, cervical spondylosis or lumbar spine lesions, pain) were not included in the analysis of QMGS.

Modified PIS Classification

We made a flowchart of the modified PIS classification based on the principle of Mutually Exclusive Collectively Exhaustive (MECE) by combining the impact categories and relevant intervals between assessment, fatigue test, PB dosage, and immunotherapies (**Figure 1**). The included patients were classified according to this flowchart into 3 categories: (1) remission (R): no symptoms, no impact on daily living, normal fatigue test (slightly incomplete eye closure is allowed), with or without immunotherapies, but without PB. (2) minimal manifestation (MM): no or mild symptoms, no impact on daily living for most of the time, abnormal fatigue test (some patients reported mild symptoms but with normal fatigue test at assessment were included in this category), with or without immunotherapies, with or without PB. MM is further divided into MM-0, MM-1, MM-2, and MM-3 according to the definition of PIS (2). (3) Slight impact (SI): same as MM except for symptoms and slight impact on daily living for most of the time. Since all included patients were mild at baseline by their self-reported impact on daily living, and there was no consensus on the quantitative definition of U and I, this category included some patients who would be classified as U and I categories. The original PIS definition of MGFA requires a minimum of 1 year for the definition of the R and MM status. In this study, various definitions of PIS were set without considering the intervals between assessment and with considering intervals of 3, 6, and 12 months, to reflect the real-time and sustained PIS through the scheduled follow-ups.

Statistical Analysis

Real-time and sustained R, MM, and SI status were recorded in patients with qualified data at baseline and the three follow-ups. Patient characteristics, treatment, and QMGS, MG-ADL, and MG-QOL15 of the patients belonging to the three categories on each follow-up were described. For quantitative data, the Kruskal–Wallis test was used for comparison among multiple groups, and the Mann–Whitney U test was used for comparison between two groups. For categorical data, the chi-square test or Fisher's exact test was used for comparison. A sensitivity cohort was established by including patients with all eligible follow-ups and relevant data of PIS categories. Optimal cutoffs between R/MM and SI were generated with the receiver operating characteristic (ROC) curve. A two-tailed $p < 0.05$ was considered statistically significant. SPSS 23.0 software was used for statistical analysis.

RESULTS

General Characteristics of Enrolled Patients

According to inclusion and exclusion criteria based on impacts on daily living, a total of 442 patients were included and

TABLE 1 | Patients belonging to each PIS category at baseline and each follow-up in the whole cohort and the sensitivity analysis cohort (number, %).

	Baseline	3 months		6 months		12 months	
		Real-time	Sustained	Real-time	Sustained	Real-time	Sustained
W cohort	***n* = 387**	***n* = 288**		***n* = 226**		***n* = 166**	
R	55 (14.2%)	88 (30.6%)	36 (12.5%)	86 (38.1%)	30 (13.3%)	62 (37.3%)	20 (12.1%)
MM	209 (54.0%)	170 (59.1%)	110 (38.2%)	120 (53.1%)	70 (31.0%)	91 (54.8%)	44 (26.5%)
MM-0	37 (9.6%)	6 (2.1%)	5 (1.7%)	0	0	0	0
MM-1	112 (28.9%)	134 (46.6%)	67 (23.3%)	111 (49.1%)	50 (22.1%)	81 (48.8%)	32 (19.3%)
MM-2	1 (0.3%)	0	0	0	0	0	0
MM-3	59 (15.2%)	30 (10.4%)	38 (13.2%)	9 (4.0%)	20 (8.8%)	10 (6.0%)	12 (7.2%)
SI	112 (28.9%)	19 (6.6%)	NA	16 (7.1%)	NA	11 (6.6%)	NA
MSI	0	5 (1.7%)	NA	2 (0.9%)	NA	2 (1.2%)	NA
I	NA	NA	56 (19.4%)	NA	57 (25.2%)	NA	42 (25.3%)
II	6 (1.6%)	5 (1.7%)	1 (0.3%)	1 (0.4%)	0	0	0
III	NA	NA	60 (20.8%)	NA	50 (22.1%)	NA	47 (28.3%)
IV	5 (1.3%)	1 (0.3%)	1 (0.3%)	1 (0.4%)	1 (0.4%)	0	0
R/MM	264 (68.2%)	258 (89.7%)	193 (67.0%)	206 (91.2%)	147 (65.0%)	153 (92.1%)	101 (60.8%)
Interval*	NA	NA	69 (24.0%)	NA	60 (26.5%)	NA	52 (31.3%)
SA cohort	***n* = 151**	***n* = 151**		***n* = 151**		***n* = 151**	
R	28 (18.5%)	51 (33.8%)	25 (16.5%)	58 (38.4%)	23 (15.2%)	58 (38.4%)	20 (13.2%)
MM	75 (49.7%)	89 (58.9%)	55 (36.4%)	82 (54.3%)	44 (29.1%)	81 (53.7%)	38 (25.2%)
MM-0	4 (2.6%)	0	0	0	0	0	0
MM-1	49 (32.5%)	76 (50.3%)	36 (23.8%)	76 (50.3%)	31 (20.5%)	72 (47.7%)	27 (17.9%)
MM-2	0	0	0	0	0	0	0
MM-3	22 (14.6%)	13 (8.6%)	19 (12.6%)	6 (4.0%)	13 (8.6%)	9 (6.0%)	11 (7.3%)
SI	47 (31.1%)	9 (6.0%)	NA	8 (5.3%)	NA	10 (6.6%)	NA
MSI	0	0	NA	2 (1.3%)	NA	2 (1.3%)	NA
I	NA	NA	27 (17.9%)	NA	36 (23.8%)	NA	38 (25.2%)
II	1 (0.7%)	2 (1.3%)	1 (0.7%)	1 (0.7%)	0	0	0
III	NA	NA	34 (22.5%)	NA	38 (25.2%)	NA	43 (28.5%)
IV	0	0	0	0	0	0	0
R+MM	103 (68.2%)	140 (92.7%)	102 (67.5%)	140 (92.7%)	96 (63.6%)	139 (92.1%)	89 (58.9%)
Interval*	NA	NA	39 (25.8%)	NA	45 (29.8%)	NA	50 (33.1%)

*R, remission; MM, minimal manifestation; SI, slight impact on daily living; MSI, moderate/serious impact on daily living; NA, not applicable; W cohort, whole cohort; SA cohort, sensitivity analysis cohort. *Interval: not the same as those of I and III, but as the intervals for sustained MM+R status.*

followed. A total of fifty-five patients were excluded: 2 patients due to conflicting scores of ADL and QOL because of poor understanding of the scales, 1 patient due to pre-existing severe depression, 28 patients due to incomplete information (12 missing most of the key information, 3 with questionable QMGS records, 13 missing the impact categories at $\geq$ half of their scheduled follow-ups), 4 patients due to ambiguous impact categories, 18 patients cases due to not meeting the target impact categories at baseline when inspected retrospectively, and 2 patients whose MG diagnosis was excluded. A total of 387 patients were included in the final analysis. Among them, there were 169 males and 218 females, the current age on inclusion ranged from 15 to 87 years (48.7 $\pm$ 16.1 years), and the disease duration ranged from 7 days to 41 years (median 28.5 months, interquartile range 76 months). The onset age was 44.2 $\pm$ 18.4 years (41.8 $\pm$ 19.2 years for females and 48.8 $\pm$ 17.2 years for males).

At baseline, 376 patients could be classified into three categories, including 55 as R (14.2%), 209 as MM (54.0%), and 112 as SI (28.9%) (**Table 1**). The unclassified patients and the reasons are shown in **Table 1**. There was no difference in age, gender, and disease duration among patients belonging to the three categories. The proportion of patients with pure ocular involvement was higher in MM patients than in SI patients and was higher in MM-0–1 patients than in MM-2–3 patients. The proportion of treatment-naive (never being treated with immune therapies) patients was similar in R, MM, and SI patients (**Table 2**). There were no differences in baseline CS dosage between the MM patients and SI patients, but significantly higher than in R patients, and significantly higher in MM-2–3 patients than in MM-0–1 patients. There was no difference in the proportion of patients with IS usage among patients of the three categories, but significantly higher in MM-2–3 patients than in MM-0–1 patients. The proportion

TABLE 2 | Generalized characteristics of patients belonging to each PIS category as the baseline.

	R ($n = 55$)	MM ($n = 209$)	SI ($n = 112$)	MM 0-1 ($n = 149$)	MM 2-3 ($n = 60$)
Current age	50.0 ± 19.9	48.8 ± 15.0	47.9 ± 15.6	48.0 ± 14.4	50.8 ± 16.6
Age at onset	45.7 ± 21.4	44.5 ± 17.6	43.0 ± 17.5	43.1 ± 17.3	47.0 ± 18.0
Female (%)	52.7% (29/55)	55.0% (115/209)	56.3% (63/112)	52.3% (78/149)	38.3% (23/60)
Duration (months)	26.0 (11.0–74.0)	24.0 (4.0–83.5)	37.5(14.0–109.8)	23.0 (4.5–97.0)	27.5 (3.0–60.8)
Current ocular type (%)	0	38.3% (80/209)	22.3% (25/112)*[a]	47.0% (70/149)	16.7% (10/60)**
Treatment naive (%)	3.6% (2/55)	12.0% (25/209)	14.3% (16/112)	16.8% (25/149)	0**
Duration of treatment naive (months)	22 &1	2.0 (1.0–9.0)	6.0 (0–26.0)*[b]	2.0 (1.0–9.0)	NA

*PIS, post-intervention status; R, remission; MM, minimal manifestation; SI, slight impact on daily living. NA, not applicable. *Comparison among R, MM and SI groups (between MM and SI groups in the proportion of patients with ocular type) revealed significant difference, $P < 0.05$. [a]Significant difference between MM and SI groups, $P < 0.05$. [b]Significant difference between MM and SI groups, $P < 0.05$. **Significant difference between MM-0–1 and MM-2–3 groups, $P < 0.05$.*

TABLE 3 | The CS dosage and proportion of patients with IS or PB in patients belonging to each real-time PIS category at baseline and each follow-up.

	R	MM	SI	MM-0–1	MM-2–3
CS dose (mg)					
Baseline	15.0 (10.0–30.0)	40.0 (21.3–60.0)	50.0 (28.8–60.0)	35.0 (15.0–55.0)	55.0 (40.0–60.0)
($n = 299$)	($n = 42$)	($n = 168$)	($n = 89$)	($n = 111$)	($n = 57$)
3 months	25.0 (15.0–35.0)*	37.5 (25.0–46.3)*	38.2 ± 16.8	35.0 (25.0–45.0)	50.0 (37.5–60.0)
($n = 259$)	($n = 78$)	($n = 162$)	($n = 19$)	($n = 133$)	($n = 29$)
6 months	15.0 (10.0–20.0)*	25.0 (15.0–30.0)*	30.0 (17.5–50.0)	22.5 (15.0–30.0)*	31.1 ± 12.4*
($n = 212$)	($n = 79$)	($n = 118$)	($n = 15$)	($n = 109$)	($n = 9$)
12 months	10.0 (7.5–15.0)*	15.0 (10.0–30.0)*	20.0 (15.0–25.0)	15.0 (10.0–25.0)*	30.0 (20.0–47.5)
($n = 158$)	($n = 58$)	($n = 89$)	($n = 11$)	($n = 80$)	($n = 9$)
IS %					
Baseline	27.3% (15/55)	29.7% (62/209)	25.9% (29/112)	24.1% (36/149)	43.3% (26/60)
3 months	23.9% (21/88)	35.9% (61/170)	52.6% (10/19)*	31.4% (44/140)	56.7% (17/30)
6 months	23.3% (20/86)	48.3% (58/120)*	56.3% (9/16)	45.9% (51/111)*	77.8% (7/9)
12 months	25.8% (16/62)	52.7% (48/91)	36.4% (4/11)	48.1% (39/81)	90.0% (9/10)
PB %					
Baseline	0.0% ($n = 55$)	28.7% (60/209)	44.6% (50/112)	0.0% ($n = 149$)	100% ($n = 60$)
3 months	0.0% ($n = 88$)	17.6% (30/170)*	52.6% (10/19)	0.0% ($n = 140$)	100% ($n = 30$)
6 months	0.0% ($n = 86$)	7.5% (9/120)*	43.8% (7/16)	0.0% ($n = 111$)	100% ($n = 9$)
12 months	0.0% ($n = 62$)	11.0% (10/91)	36.4% (4/11)	0.0% ($n = 81$)	100% ($n = 10$)

*CS, corticosteroid; IS, immunosuppressant; PB, pyridostigmine bromide; PIS, post-intervention status. *Significant difference in CS dose, IS % and PB % compared with the former follow-up in patients belonging to the same PIS category, $P < 0.05$.*

of patients with PB usage was lower in MM patients than in SI patients (**Table 3**).

PIS Categories at Different Follow-Ups

In the whole cohort, the proportion of patients belonging to each real-time PIS category at baseline and 3, 6, and 12 months is shown in **Table 1**. 14.2–38.1% of the patients were classified as R, with a significant increase at 3 months compared with the baseline, and little change thereafter; 53.1–59.0% were classified as MM, with a slight increase at 3 months compared with the baseline, and little change thereafter; 6.6–28.9% were classified as SI, with a significant decrease at 3 months compared with the baseline, and little change thereafter. Only a small number of patients developed MSI (0.9–1.7%). 0.8–2.9% could not be classified, because the PB dosage did not meet the requirement for R (II) or MM-2 (IV) by the PIS definition, which was mainly observed at baseline ($n = 11$) and 3 months ($n = 6$).

The proportion of patients belonging to each sustained PIS category at 3, 6, and 12 months is shown in **Table 1**. 12.1–13.3% were classified as R, and 26.5–38.2% as MM, with no significant difference at the three follow-ups. 40.8–53.6% of the patients could not be classified, which slightly increased at 3 months compared with the baseline, with little change thereafter. The main reason is that the status cannot sustain to cover the requested intervals (I and III) (**Table 1**).

Since the whole cohort included the patients whose follow-up time was less than 12 months (loss of follow-up, or not yet reached 12 months due to the principal investigator moving from

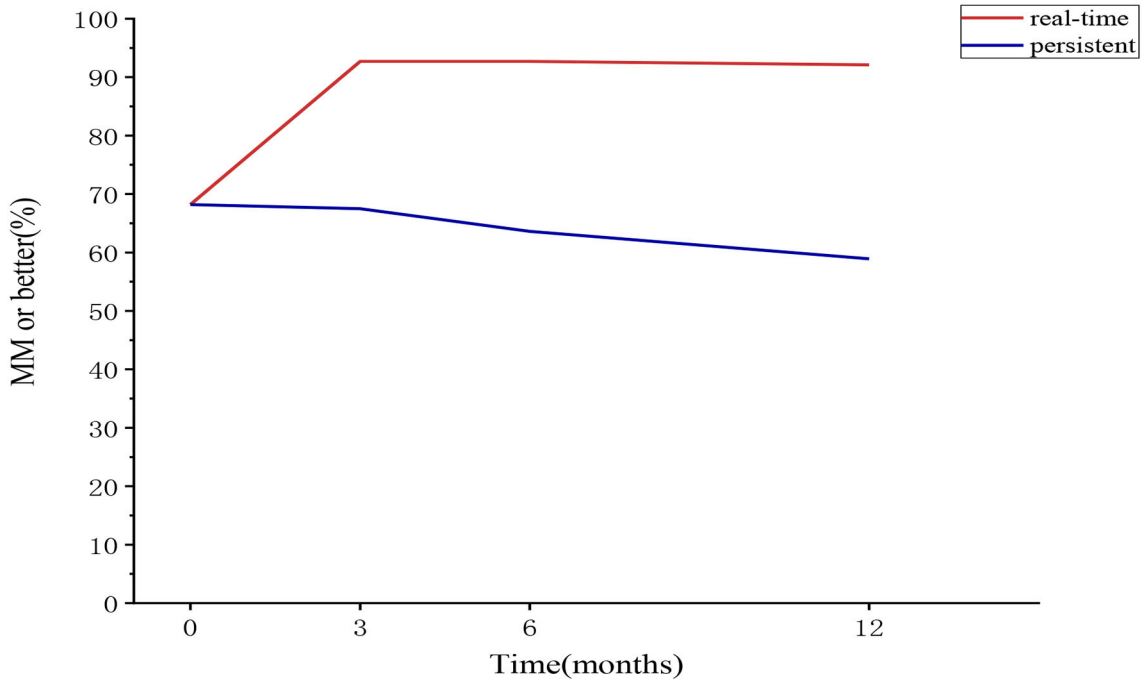


FIGURE 2 | The proportion of patients belonging to the "MM or better" category at each follow-up in the sensitivity analysis cohort (real-time and sustained). MM, minimal manifestation.

Qilu Hospital to Xuanwu Hospital) and patients whose impact category was missing at some follow-ups, we selected the patients who had all impact category information at the baseline and 3 follow-ups to establish a sensitivity analysis (SA) cohort. The proportion of patients belonging to each PIS category at baseline and each follow-up in this cohort was similar to those of the whole cohort. Only two patients were unable to be classified due to the PB dosage. The patients who could not be classified due to the requested intervals by PIS definition were similar in both cohorts (**Table 1**).

The proportion of patients belonging to each real-time or sustained R/MM (MM or better) status at 3, 6, and 12 months was 89.7–92.1% and 60.8–67%, with no significant difference at the three follow-ups. About 0.8–2.9% and 25−31.3% of the patients could not be classified for each real-time or sustained R/MM status, with little change after 3 months (**Table 1** and **Figure 2**).

Changes of Treatment and Real-Time PIS Categories at Different Follow-Ups

The CS dosage in real-time R patients increased at 3 months, which is mainly related to the transfer of patients in the former MM and SI categories to this category. It fell to the baseline level at 6 months and further decreased at 12 months. There was decreasing trend in the CS dosage in the MM category at 3, 6 and 12 months compared with the former follow-ups. The CS dosage in the SI category showed an overall decreasing trend, although there was no difference in each follow-up compared with the previous one. The overall trend of CS dosage in the MM-0–1 sub-category was the same as that in the R category, although the CS dosage was larger than that in R category. The CS dosage in the MM-2–3 sub-category showing an overall decreasing trend, with little change after 6 months. There was no difference in the proportion of concurrent IS usage in the R category. The IS usage in the MM category at 6 and 12 months was higher than the baseline and 3 months. The IS usage in the SI category at 3 months was higher than the baseline and decreased after 6 months. The overall trend of IS usage in the MM-0–1 sub-category was the same as that of the MM category, and the overall trend of that in the MM-2–3 sub-category showed an increasing trend. The proportion of concurrent PB usage in the MM category was lower at 3 and 6 months than that of the previous follow-up, and there was no difference between 6 and 12 months. The overall trend of PB usage in the SI category decreased. There was no difference in PB usage at different follow-ups in the R, MM-0–1 and MM-2–3 categories (**Table 3**).

In the whole cohort, 68.8–89.7%, 71–76.7%, and 19.8–77.1% of the patients classified in real-time R, MM, and SI categories remained unchanged in each follow-up compared with the previous follow-up, respectively. The proportions of patients in MM category and SI category who remained unchanged at each follow-up were similar, except for the SI category which had a significant decrease at 3 months compared with the baseline. The proportions of patients in the sensitivity analysis cohort who remained unchanged at each follow-up were similar to those in the whole cohort (**Table 4**).

Since the proportions of patients who could be classified as R and MM and patients who remained unchanged in the R and MM categories were similar in the two cohorts (**Tables 1**, **4**), the

TABLE 4 | Changes in patients belong to each real-time PIS category compared with the former follow-ups in the whole cohort and sensitivity analysis cohort.

Baseline	R			MM			SI		
PIS	No change	Improvement	Worsening	No change	Improvement	Worsening	No change	Improvement	Worsening
Whole cohort									
3 months	89.7% (35/39)	NA	10.3% (4/39)	71.0% (110/155)	26.4% (41/155)	2.6% (4/155)	19.8% (17/86)	76.7% (66/86)	3.5% (3/86)
6 months	78.9% (30/38)	5.3% (2/38)	15.8% (6/38)	76.7% (92/120)	13.3% (16/120)	10.0% (12/120)	76.9% (50/65)	20.0% (13/65)	3.1% (2/65)
12 months	68.8% (22/32)	15.6% (5/32)	15.6% (5/32)	72.9% (62/85)	14.1% (12/85)	12.9% (11/85)	77.1% (37/48)	6.3% (3/48)	16.6% (8/48)
Sensitivity analysis cohort									
3 months	89.3% (25/28)	NA	10.7% (3/28)	73.3% (55/75)	25.3% (19/75)	1.4% (1/75)	19.1% (9/47)	80.9% (38/47)	0
6 months	82.1% (23/28)	3.6% (1/28)	14.3% (4/28)	81.3% (61/75)	10.7% (8/75)	8.0% (6/75)	80.9% (38/47)	14.9% (7/47)	4.3% (2/47)
12 months	75.0% (21/28)	10.7% (3/28)	14.3% (4/28)	74.7% (56/75)	10.7% (8/75)	14.6% (11/75)	78.7% (37/47)	6.4% (3/47)	14.9% (7/47)

PIS, post-intervention status; R, remission; MM, minimal manifestation; SI, slight impact on daily living; NA, not applicable.

subsequent analysis of the QMGS, MG-ADL, and MG-QOL15 scores used the data from the whole cohort.

Changes in Sustained PIS Categories at Different Follow-Ups

Using the data of the sensitivity analysis cohort, the sustainability of the PIS was explored (**Table 5**). About 87–92% of the patients belonging to the baseline R category remained in R category, with no difference through 3, 6, and 12 months; 73.3–86.4% of the patients belonging to the baseline MM category remained in the MM category, with the increasing trend through 3, 6, and 12 months; 92.7–99% of the patients belonging to the baseline R/MM category remained in the R/MM category, with no difference through 3, 6, and 12 months. Among the patients with changes in PIS categories, there were continuous improvements, improvement after initial worsening, or worsening after initial remission (**Table 5**). At least 86.4% (89/103) of the baseline R/MM patients remained in R/MM status at all follow-ups. The proportion of patients belonging to real-time and sustained R/MM status in the sensitivity analysis cohort is shown in **Figure 2**.

The real-time (6 or 12 months) R/MM status in the sensitivity analysis cohort of patients with sustained or non-sustained R/MM status of different intervals (3 or 6 months) is shown in **Table 6**. Whether there was sustained or non-sustained R/MM status in these patients, there was no difference in the proportion of attaining real-time R/MM status at 6 or 12 months.

QMGS, MG-ADL, and MG-QOL15 Scores in Patients Belonging to Each Category at Different Follow-Ups

The patients with all data of QMGS, MG-ADL, and MG-QOL15 scores were used for quantification of each PIS category in the whole cohort. There were significant differences in the QMGS, MG-ADL, and MG-QOL15 scores (by mean ± SD or interquartile range) among patients belonging to each real-time category (R, MM, and SI), which were consistent at baseline and 3, 6, and 12 months of follow-ups. The three scores ranked from small to large as in the following categories R < MM <SI. The QMGS and MG-QOL15 scores were significantly lower in patients belonging to the MM-0–1 sub-category than those in the patients belonging to the MM-2–3 sub-category, whereas no differences were found in the MG-ADL scores between patients belonging to the two sub-categories. Although there was a significant decrease in some scores at 3 months compared with baseline, the overall trend of no significant changes was noted in patients belonging to the same category at each follow-up (**Table 7** and **Figure 3**). The same trend was observed in patients belonging to each sustained PIS category during the follow-up, with smaller values than the same items of real-time categories and little difference between the MM-0–1 sub-category and MM-2–3 sub-category. A significant decrease in trend was noted in sustained MM-2–3 sub-categories than those of real-time MM-2–3 sub-categories.

TABLE 5 | Changes in patients belonging to each sustained PIS category compared with the former follow-ups in the sensitivity analysis cohort.

	R					MM				R/MM			
	U	I	W			U	I	W		U	I	W	
	R	NA	MM	SI	MSI	MM	R/II	SI	MSI	R/MM	NA	SI	MSI
3 mon	89.3% (25/28)	NA	10.7% (3/28)	0	0	73.3% (55/75)	25.3% (19/75)	1.4% (1/75)	0	99.0% (102/103)	NA	1.0% (1/103)	0
6 mon	92.0% (23/25)	NA	8.0% (2/25)	0	0	80.0% (44/55)	12.7% (7/55)	3.6% (2/55)	3.6% (2/55)	94.1% (96/102)	NA	3.9% (4/102)	2.0% (2/102)
12 mon	87.0% (20/23)	NA	13.0% (3/23)	0	0	86.4% (38/44)	6.8% (3/44)	6.8% (3/44)	0	92.7% (89/96)	NA	5.2% (5/96)	2.1% (2/96)

PIS, post-intervention status; U, unchanged; I, improvement; W, worsening; NA, not applicable; R, remission; MM, minimal manifestation; SI, slight impact on daily living; MSI, moderate or serious impact on daily living.

TABLE 6 | Sustained intervals of R/MM status and the real-time R/MM status at each follow-up in the sensitivity analysis cohort.

	Sustained R/MM at 3 months ($n = 102$)	Non-sustained R/MM at 3 months ($n = 1$)	Sustained R/MM at 6 months ($n = 96$)	Non-sustained R/MM at 6 months ($n = 7$)
R/MM at 6 months	96[a]	1		
Non-R/MM at 6 months	6	0		
R/MM at 12 months	97[b]	1	89[c]	7
Non-R/MM at 12 months	5	0	7	0

R, remission; MM, minimal manifestation. [a]$p = 1.00$; [b]$p = 1.00$; [c]$p = 1.00$.

The range of the scores was larger in some patients. For example, we used the real-time QMGS > 12 to show the proportion of patients with higher scores. At baseline, 12 out of 157 MM patients had higher QMGS (13–20), and their disease duration was 15–290 months. At 3 months, 5 out of 110 MM patients had higher QMGS (13–18), with a duration of 24–179 months. At 6 months, 3 out of 79 MM patients had higher QMGS (all 16), with a duration of 11–116 months. And at 12 months, 5 out of 75 MM patients had higher QMGS (13–18), with a duration of 74–197 months.

The cutoffs of QMGS, MG-ADL, and MG-QOL15 scores between the R/MM and SI status were generated with the ROC curve. The sensitivity was 76.8–100% and the specificity was mostly 61.4–88.5% (**Table 8**).

DISCUSSION

To our knowledge, this is the first study to systematically follow the changes in PIS and relevant QMGS, MG-ADL, and MG-QOL15 scores in patients who reported no or slight impacts of their symptoms on daily living.

For the sustainability of PIS in these patients whose current severity was at the milder end of the disease spectrum, this study is aimed to explore whether commonly used severity scores can provide a quantitative reference for these statuses.

The baseline PIS categories and proportion of treatment-naive were representative. The sustainability of PIS was examined and analyzed along with treatment changes. For the real-time PIS categories, there was an increase in the R and MM patients and a significant decrease in SI patients during the initial 3 months. At baseline, 2.9% of the patients could not be classified due to the usage of PB and PB dosage beyond the definition of relevant PIS categories. The CS dosage in R patients increased due to the conversion of former MM or SI patients into R patients accordingly. The CS dosage in MM and SI patients was on the decreasing trend. The proportion of patients on IS was stable in R patients and increased in MM and SI patients. The proportion of patients on PB decreased in MM and SI patients, especially in MM patients, however, the decrease was

TABLE 7 | The scores of QMG, MG- ADL, and QOL in patients belonging to each PIS category at each follow-up in the whole cohort (real-time or sustained).

	R	*n*	R/MM	*n*	MM	*n*	SI	*n*	MM-0–1	*n*	MM-2–3	*n*
Real-time												
Baseline												
QMGs ($n = 274$)	0 (0–0.03)	35	3.33 (1.33–6.65)	192	3.99 (2.66–7.98)	1,57	7.98 (5.24–12.97)*	82	3.82 (2.66–5.49)	118	7.98 (3.99–11.97)**	39
ADL ($n = 274$)	0 (0–2.00)	35	1.50 (0–3.00)	192	2.00 (0–3.00)	157	3.66 ± 2.24*	82	2.00 (0.75–3.00)	118	2.00 (0–3.00)	39
QOL ($n = 274$)	3.00(0–5.00)	35	5.00 (2.00–10.00)	192	6.00 (2.00–11.00)	157	13.00 (7.00–21.00)*	82	6.00 (2.00–10.00)	118	9.00 (3.0–12.00)**	39
3 months												
QMGS ($n = 182$)	0 (0–0.03)	61	2.66 (0–4.99)†	171	3.99 (2.66–6.65)	110	7.98 (2.66–9.31)*	11	3.66 (2.66–6.65)	86	5.99 (2.99–9.31)**	24
ADL ($n = 182$)	0 (0–1.00)	61	1.00 (0–2.00)†	171	1.00 (0–3.00)†	110	2.82 ± 1.78*	11	1.00 (0–2.00)†	86	1.5 (0–3.00)	24
QOL ($n = 182$)	1.00 (0–4.00)	61	3.00 (0–7.00)†	171	4.00 (1.00–9.00)†	110	8.82 ± 6.60*†	11	3.50 (1.00–8.00)†	86	7.00 (3.00–13.00)**	24
6 months												
QMG ($n = 139$)	0 (0–0.02)	55	1.33 (0–5.07)	134	3.99 (2.66–6.65)	79	7.98 (7.32–11.81)*	5	3.99 (2.66–6.65)	75	8.48 (3.58–14.38)	4
ADL ($n = 139$)	0 (0)	55	0 (0–1.00)†	134	1.00 (0–2.00)	79	3.00 (2.00–4.50)*	5	1.00 (0–2.00)	75	0.50 (0–3.25)	4
QOL ($n = 139$)	0 (0–3.00)†	55	2.50 (0–5.00)	134	4.00 (1.00–7.00)	79	10.00 (6.50–18.00)*	5	4.0 (1.00–7.00)	75	3.50 (0.50–17.00)	4
12 months												
QMG ($n = 139$)	0 (0–0.02)	57	1.33 (0–3.99)	132	3.33 (2.66–6.32)	75	7.98 (6.65–10.98)*	7	2.66 (1.33–5.32)	66	5.32 (3.66–15.30)**	9
ADL ($n = 139$)	0 (0–0.50)	57	0 (0–1.00)	132	0 (0–2.00)	75	2.00 (2.00–6.00)*	7	0 (0–2.00)	66	2.00 (0–3.50)	9
QOL ($n = 139$)	0 (0–2.00)	57	2.00 (0–5.00)	132	4.00 (1.00–6.00)	75	8.00 (5.00–9.00) *	7	3.00 (1.00–6.00)	66	7.89 ± 3.98**	9
Sustained												
3 months												
QMGS ($n = 125$)	0 (0–0.03)	23§	1.33 (0–3.99)†	125	3.66 (1.33–5.32)†	67§	NA		2.66 (1.33–3.99)	39	4.83 (2.66–6.65)**†	28
ADL ($n = 125$)	0 (0–1.00)	23§	0 (0–2.00)†	125	1.00 (0–3.00)†	67§	NA		1.00 (0–3.00)†	39	1.00 (0–3.00)	28
QOL ($n = 125$)	0 (0–3.00)†	23§	3.00 (0–6.00)†	125	4.00 (1.00–8.00)†	67§	NA		3.00 (1.00–8.00)†	39	4.00 (1.25–10.75)†	28
6 months												
QMGS ($n = 96$)	0 (0–0.02)	21§	1.17 (0–3.66)	96	2.66 (1.33–6.24)	44§	NA		3.66 (2.33–6.65)	35	2.66 (1.33–4.49)†	9
ADL ($n = 96$)	0 (0)†	21§	0 (0–1.00)†	96	1.00 (0–2.00)	44§	NA		1.00 (0–2.00)	35	1.00 (0–1.50)	9
QOL ($n = 96$)	0 (0–1.50)	21§	2.00(0–5.00)	96	3.00 (1.00–6.75)	44§	NA		3.00 (0–6.00)	35	3.00 (2.00–9.50)	9
12 months												
QMGS ($n = 87$)	0 (0–0.02)	19§	1.00 (0–2.66)	87	2.66 (1.33–5.32)	35§	NA		2.66 (1.33–5.32)	25	2.66 (1.33–4.32)	10
ADL ($n = 87$)	0 (0)	19§	0 (0–1.00)	87	0 (0–2.00)	35§	NA		0 (0–2.00)	25	0.00 (0–1.00)	10
QOL ($n = 87$	0 (0–1.00)	19§	1.00 (0–4.00)	87	3.00 (1.00–5.00)	35§	NA		3.00 (1.00–5.00)	25	3.00 (1.00–5.75)	10

*Mean ± standard deviation (SD) for data of normal distribution, median (interquartile range) for data of abnormal distribution. QMGS, quantitative myasthenia gravis score; ADL, activities of daily living; QOL, quality of life; PIS, post-intervention status; R, remission; MM, minimal manifestation; SI, slight impact on daily living; NA, not applicable. *Comparison among R category, MM category and SI category at the same follow-up, $p < 0.05$. **Comparison between MM-0–1 category and MM-2–3 category at the same follow-up, $p < 0.05$. †Comparison between each follow-up and the former follow-up in the same category, $p < 0.05$. §The sum number of patients belonging to sustained R and MM categories were not equal to the number of patients belonging to sustained R/MM status due to R/MM contained the patients converted between the two categories.*

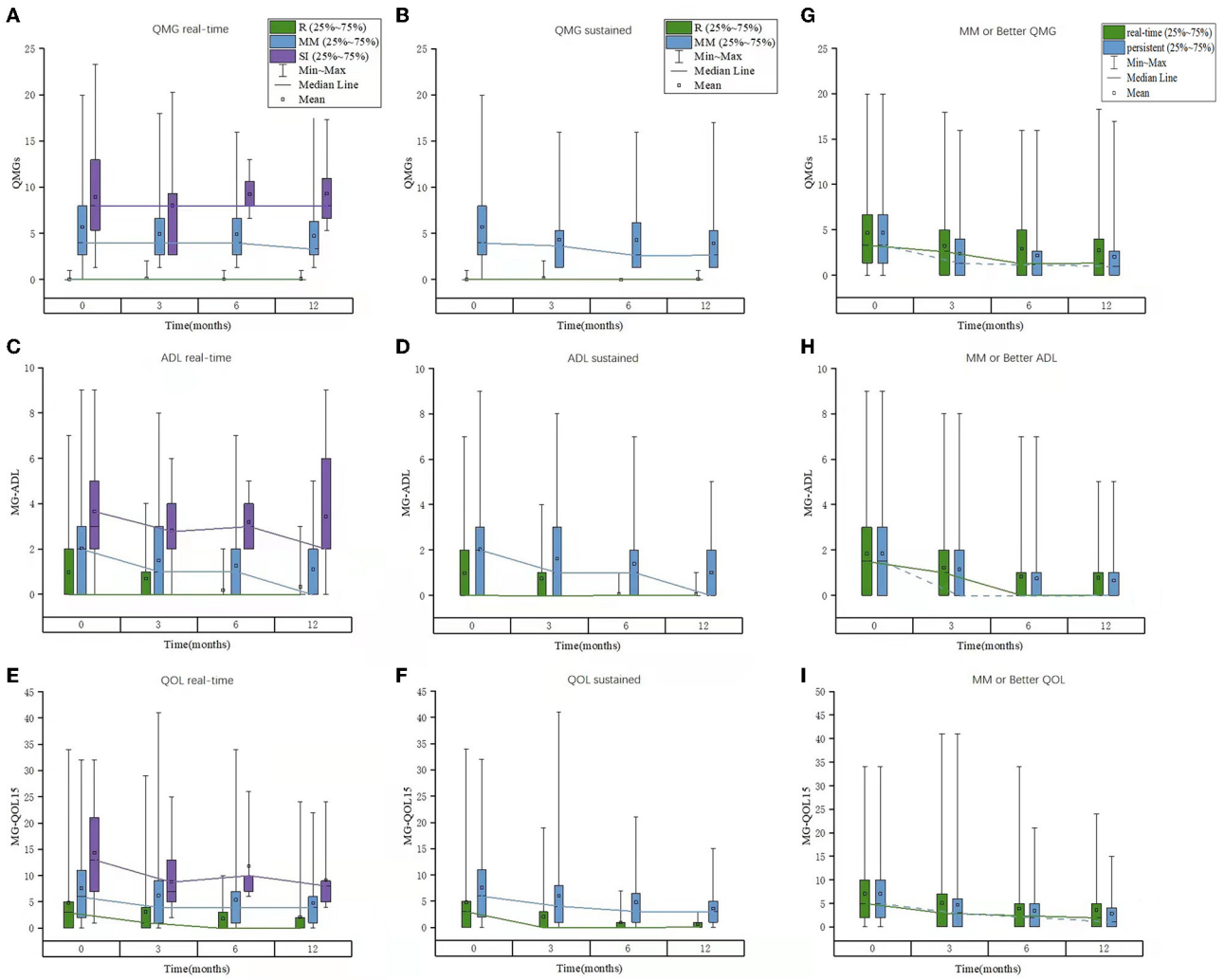


FIGURE 3 | The scores of QMGS, MG-ADL, and MG-QOL15 in patients belonging to each PIS category or "MM or better" status at each follow-up in the whole cohort. **(A–C)** Real-time; **(D–F)** Sustained; **(E–I)** "MM or better" status. QMGS, quantitative myasthenia gravis score; ADL, activities of daily living; QOL, quality of life; PIS, post-intervention status; MM, minimal manifestation.

only seen in MM-0–1 patients. Larger CS dosage and more IS and PB were used in MM-2–3 and SI patients compared with MM-0–1 and R patients. These indicated that some patients with more severe PIS may convert into milder PIS along with decreased SC dosage and PB usage with the aid of adding IS and some patients will continue to improve despite the tapering of treatments. However, some patients may still need intense immunological treatment. In our practice, we encouraged patients to taper and withdraw PB after improvement as early as possible. In this cohort, with the extension of follow-up, the patients had a better understanding of reducing PB dosage after improvement. The PB dosage did not meet the PIS definition in only 2 patients at 6 months and none of the patients at 12 months.

For the sustained PIS categories, there was a similar proportion of R and MM, with a slightly decreasing trend. Half of the patients could not be classified, mainly due to the conversion of PIS, including continuous improvement, improvement after initial worsening, or worsening after initial remission. Due to the sustainability of R and MM being poor, MM or better R/MM was analyzed in both cohorts. The sustained R/MM status was found in at least 60.8% and 58.9% of the patients in the whole and SA cohorts. The sustainability of the R, MM, and R/MM status in the SA cohort was similar to that in the whole cohort, indicating that a close follow-up could not change the PIS categories.

To further explore the sustainability of PIS, we analyzed the unchanged patients belonging to baseline R and MM in the SA cohort. In unchanged patients at 3 months, the proportion of being unchanged in the next follow-up was high, indicating a stable state in these patients. At least 86.4% of the baseline R/MM patients remained in the R/MM status at

TABLE 8 | Cutoffs of QMGS, MG-ADL, and MG-QOL15 between "MM or better" and SI at each follow-up in the whole cohort.

	AUC (95%CI)	*P*-Value	Optimal cut-offs	Sensitivity	Specificity	Accuracy
Real-time						
Baseline						
QMGS (*n* = 274)	0.756 (0.698–0.815)	<0.01	4.83	76.8%	64.1%	67.9%
ADL (*n* = 274)	0.737 (0.673–0.801)	<0.01	1.50	85.4%	50.0%	60.6%
QOL (*n* = 274)	0.771 (0.714–0.828)	<0.01	5.50	90.2%	50.5%	62.4%
3 months						
QMGS (*n* = 182)	0.809 (0.705–0.914)	<0.01	2.33	100%	49.7%	52.7%
ADL (*n* = 182)	0.769 (0.636–0.901)	<0.01	1.50	81.8%	67.8%	68.7%
QOL (*n* = 182)	0.725 (0.610–0.839)	0.013	4.50	81.8%	61.4%	62.6%
6 months						
QMGS (*n* = 139)	0.924 (0.868–0.980)	<0.01	6.49	100%	81.3%	82.0%
ADL (*n* = 139)	0.904 (0.833–0.974)	<0.01	1.50	100%	77.6%	78.4%
QOL (*n* = 139)	0.893 (0.818–0.969)	<0.01	5.50	100%	76.1%	77.0%
12 months						
QMGS (*n* = 139)	0.914 (0.859–0.969)	<0.01	4.99	100%	80.3%	81.3%
ADL (*n* = 139)	0.821 (0.649–0.996)	<0.01	1.50	85.7%	79.5%	80.0%
QOL (*n* = 139)	0.824 (0.734–0.913)	<0.01	3.50	100%	64.4%	66.2%
Sustained						
3 months						
QMGS (*n* = 125)	0.824 (0.734–0.913)	<0.01	3.50	100%	64.4%	72.1%
ADL (*n* = 125)	0.777 (0.646–0.908)	<0.01	1.50	81.8%	69.6%	70.6%
QOL (*n* = 125)	0.752 (0.640–0.863)	<0.01	4.50	81.8%	66.4%	67.6%
6 months						
QMGS (*n* = 96)	0.954 (0.911–0.997)	<0.01	6.49	100%	88.5%	89.1%
ADL (*n* = 96)	0.910 (0.844–0.977)	<0.01	1.50	100%	81.3%	82.2%
QOL (*n* = 96)	0.896 (0.816–0.976)	<0.01	5.50	100%	76.0%	77.2%
12 months						
QMGS (*n* = 87)	0.944 (0.895–0.993)	<0.01	4.66	100%	85.1%	86.2%
ADL (*n* = 87)	0.836 (0.665–1.000)	<0.01	1.50	85.7%	82.8%	83.0%
QOL (*n* = 87	0.876 (0.797–0.955)	<0.01	3.50	100%	72.4%	74.5%

QMG, quantitative myasthenia gravis score; ADL, activities of daily living; QOL, quality of life; MM, minimal manifestation; SI, slight impact on daily living.

12 months. Whether they were in sustained or non-sustained R/MM status at 3 or 6 months, they were still keeping the real-time R/MM status at 6 or 12 months. This indicated that the MM or better status was an indicator of a stable state of MG.

There were significant differences in the QMGS, MG-ADL, and MG-QOL15 scores among patients belonging to each real-time category (R, MM, and SI), which were consistent at baseline and 3-, 6- and 12-month follow-ups. The same trend was observed in patients belonging to each sustained PIS category during the follow-up. Moreover, the QMGS, MG-ADL, and MG-QOL15 scores were found smaller in patients of sustained PIS categories than those in patients of the same real-time categories, especially in the MM-2–3 sub-category, indicating a more stable disease state of MG in patients with sustained PIS. The optimal cutoffs between the R/MM and SI categories were satisfactory at most of the follow-ups. These facts indicated that the three commonly used scoring systems were eligible to provide a quantitative reference for the R and MM status.

In the Japanese study of PIS, the total QMGS score was reported as 2.6 ± 1.5, 3.0 ± 2.0, and 4.6 ± 2.4 and the total MG-QOL15 score was reported as 8.8 ± 9.7, 9.4 ± 10.5, and 11.5 ± 10.5 for the complete stable R, pharmacological R, and MM status (5). In the Canadian study, the total scores of QMGS, MG-ADL, and MG-QOL15 were reported as 4.28 ± 2.78, 1.04 ± 1.21, and 6.28 ± 7.45 for the patient-acceptable symptom states (6). The minimal symptom expression was defined as MG-ADL total score of 0–1 or MG-QOL15 total score of 0–3 (7) In our study, the total QMGS score was reported as 0 (0-0.03) and 3.99 (2.66–7.98), the total MG-ADL MG-ADL scores was 0 (0–2.00) and 2.00 (0–3.00), and the total MG-QOL15 score was 6.00 (2.00–11.00) for the R and MM status. The difference between the four studies might be due to the difference in details in the definition of R and MM, and the subjective experience in the MG symptoms and the severity of the included patients.

This study has its strength in its representative distribution of baseline real-time PIS categories in a real-world cohort and the large sample size. Detailed changes in PIS categories and their relation to the changes in treatment were shown by comparison through the follow-up time points. However, several limitations should be emphasized. The number of SI patients was small at the last two follow-ups due to inadequate or loss of follow-up, which might overestimate the accuracy of cutoffs between the R/MM and SI status. However, the sensitivity and specificity were intrinsic determinants of the accuracy, which was relatively satisfactory. Several patients with high QMGS, MG-ADL, and MG-QOL15 were included, which might enlarge their ranges and IQRs. This reflected the real-world conditions of the patients, whose disease duration was found to be long, which might render them to tolerate their symptoms well subjectively. Moreover, although the agreement between QMGS and MG-ADL was found good in moderate or severe MG patients (8), the correlation between the two scores was weaker in patients who were in the MM status, demonstrating a "floor effect". The disagreement was also found in mild patients in this study. We will further explore the factors associated with discordance between the physician-evaluated score (QMGS) and the self-reported MG-ADL or MG-QOL15. Furthermore, there might be effects from patients who dropped out from follow-up on the rates of PIS (particularly R status) at 12 months. However, because of the complex reasons for drop-out (due to neglect of minor fluctuation or random drop-out), a sensitivity analysis on the specific PIS categories and scores could not be conducted in light of the absence of drop-out reasons at the individual level.

In conclusion, the sustainability of R status was confirmed as poor. However, the sustainability of R/MM status was confirmed as excellent. The R/MM status indicated a stable state in MG patients. The QMGS, MG-ADL, and MG-QOL15 scores may provide a quantitative reference for these PIS.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Xuanwu Hospital, Capital Medical University; Ethics Committee of Qilu Hospital (Qingdao), Cheeloo College of Medicine, Shandong University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

H-FL conceptualized and designed the study and revised the manuscript. PJ and JL interpreted the data and wrote the manuscript. H-YL, BZ, and Y-XY assisted in the follow-up and assessment of QMGS. S-YW and X-CZ assisted in the assessment of MG-ADL and MG-QOL15. S-SL, Y-FL, and L-DJ contributed to statistical analysis and manuscript revision. H-FL and Y-XY diagnosed, treated, recruited, and followed up with these patients in this study. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the National Natural Science Foundation of China (Grant Nos. 81771362 and 82171397 to H-FL).

REFERENCES

1. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. *Neurology.* (2016) 87:419–25. doi: 10.1212/WNL.0000000000002790
2. Jaretzki A, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. *Neurology.* (2000) 55:16–23. doi: 10.1212/WNL.55.1.16
3. Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. *Muscle Nerve.* (2008) 37:141–9. doi: 10.1002/mus.20950
4. Nagane Y, Suzuki S, Suzuki N, Utsugisawa K. Early aggressive treatment strategy against myasthenia gravis. *Eur Neurol.* (2011) 65:16–22. doi: 10.1159/000322497
5. Masuda M, Utsugisawa K, Suzuki S, Nagane Y, Kabasawa C, Suzuki Y, et al. The MG-QOL15 Japanese version: validation and associations with clinical factors. *Muscle Nerve.* (2012) 46:166–73. doi: 10.1002/mus.23398
6. Mendoza M, Tran C, Bril V, Katzberg HD, Barnett C. Patient-acceptable symptom states in myasthenia gravis. *Neurology.* (2020) 95:e1617–e1628. doi: 10.1212/WNL.0000000000010574
7. Vissing J, Jacob S, Fujita KP, O'Brien F, Howard JF, REGAIN study group. 'Minimal symptom expression' in patients with acetylcholine receptor antibody-positive refractory generalized myasthenia gravis treated with eculizumab. *J Neurol.* (2020) 267:1991–2001. doi: 10.1007/s00415-020-09770-y
8. McPherson T, Aban I, Duda PW, Farzaneh-Far R, Wolfe GI, Kaminski HJ, et al. Correlation of Quantitative Myasthenia Gravis and Myasthenia Gravis Activities of Daily Living scales in the MGTX study. *Muscle Nerve.* (2020) 62:261–6. doi: 10.1002/mus.26910

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Neurology

BRIEF RESEARCH REPORT
published: 02 June 2022
doi: 10.3389/fneur.2022.880040

Criteria for Treatment Response in Myasthenia Gravis: Comparison Between Absolute Change and Improvement Percentage in Severity Scores

Hong-Yan Li[1†], Ping Jiang[2†], Yanchen Xie[3], Bing Liang[1], Ling Li[1], Cuiping Zhao[1], Yao-Xian Yue[1] and Hai-Feng Li[2*]*

[1] Department of Neurology, Qilu Hospital (Qingdao), Cheeloo College of Medicine, Shandong University, Qingdao, China, [2] Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China, [3] Department of Neurology, Beijing Friendship Hospital, Capital Medical University, Beijing, China

OPEN ACCESS

Edited by:
Xin-Ming Shen,
Mayo Clinic, United States

Reviewed by:
Hua Zhang,
Beijing Hospital, Peking University, China
Kimiaki Utsugisawa,
Hanamaki General Hosipital, Japan

***Correspondence:**
Yao-Xian Yue
yyx12550@163.com
Hai-Feng Li
drlhf@163.com

[†]*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *20 February 2022*
Accepted: *03 May 2022*
Published: *02 June 2022*

Citation:
Li H-Y, Jiang P, Xie Y, Liang B, Li L, Zhao C, Yue Y-X and Li H-F (2022) Criteria for Treatment Response in Myasthenia Gravis: Comparison Between Absolute Change and Improvement Percentage in Severity Scores. Front. Neurol. 13:880040. doi: 10.3389/fneur.2022.880040

Background: The absolute change in the severity score between the baseline and pre-specified time frame (absolute criterion) was recommended as a criterion for myasthenia gravis (MG) treatment response. But heterogeneity of disease severity might dilute major changes in individual patients. The rationality of relative criterion (improvement percentage) had not been evaluated in treatment response in patients with MG.

Objectives: To investigate the consistency between an absolute criterion and a relative criterion in the evaluation of treatment response in patients with MG.

Methods: We retrospectively analyzed the treatment response to a 3-month standardized treatment protocol with only glucocorticoid in 257 MG patients native to immunological treatments. With the commonly used absolute criterion, cut-offs of relative criteria were generated with the receiver operating characteristic (ROC) curve in the whole cohort and in patients with different degrees of baseline severity stratified by pre-treatment quantitative myasthenia gravis score (QMGS). The consistency between absolute and relative criteria was examined with Cohen's Kappa test and Venn diagrams.

Results: The absolute and relative criteria had an overall substantial consistency (Kappa value, 0.639, $p < 0.001$) in the cohort. The Kappa values were substantial to almost perfect in mild and moderate groups and moderate in severe groups between the absolute and relative criteria (all $p \leq 0.001$). More patients were classified as responsive with an absolute criterion while as unresponsive with a relative criterion in the moderate and severe groups.

Conclusions: The overall consistency between absolute and relative criteria was substantial in the whole cohort. The inconsistency between the two criteria was mainly from the moderate or severe patients at the baseline.

Keywords: myasthenia gravis, criteria, treatment response, improvement percentage, severity

INTRODUCTION

In the guideline for clinical trials of myasthenia gravis (MG), quantitative measure, such as the MG composite, was recommended for determining improvement and worsening for patients with MG. Other quantitative measures were encouraged to be validated for the same purpose. The absolute change in the severity score between the baseline and pre-specified time frame was recommended as the criterion for treatment response (1). The quantitative myasthenia gravis score (QMGS) is a validated and frequently used measure in clinical trials and observational studies. Barohn et al. reported the interrater reliability of QMGS and considered the change of QMGS of $>$ 2.6 points as clinical significance (2). In a study that assessed the responsiveness of QMGS, Bedlack (3) reported an average decrease of 2.3 points in the improved group. Minimal difference has been established for clinical trials of MG, which showed a QMGS change cut-off $\leq$ 3, was clinically important (4). However, the difference derived from group comparison is unfeasible when used in defining the responsiveness of individual patients to a given treatment. In a genetics study of glucocorticoid (GC) sensitivity, Xie et al. (5) used the definition of "improvement $\geq$ 3 points in QMGS or QMGS decreased to 0 after a 3-month GC treatment" as the criterion to analyze the factors that might be associated with the short-term sensitivity to GC.

The heterogeneity of disease severity might dilute major changes in individual patients by comparison at the group level, particularly in patients with mild and severe involvement. In our correspondence to this guideline (6), we proposed using a relative score that is based on the improvement percentage of an individual patient during the interval for treatment response evaluation. The relative score was defined as ($score_{pre-treatment} - score_{post-treatment}$)/$score_{pre-treatment}$. In China, such a relative scoring system had been used for more than 25 years (7). The relative score may provide a useful individualized evaluation of therapeutic effects and can be analyzed as a linear parameter. Furthermore, comparison of the proportions of patients in both treatment and placebo groups who met a pre-specified effect criterion based on the relative score may provide us with another view of the treatment effects, even if between-group comparisons showed no significant differences. In a genetic study on rheumatoid arthritis, in which definition of individual treatment effect was essential, a similar criterion based on improvement percentage was used (8). In reply to our correspondence (6, 9), the authors stated that skewed distributed baseline severity and relevant stratification of disease severity might lead to potential bias in using a relative score as a criterion.

Glucocorticoids are the first-line immunosuppressive treatment for MG because of their rapid effect and controllable side effects (10, 11). Large-size retrospective studies have shown significant improvement in patients with MG with different doses of GCs. The mean duration between the onset to improvement after GC treatment was 13∼14 days; the mean onset to sustained improvement was 1.5∼3 months (12). Hence, the responsiveness to GCs is a good example of a short-term treatment effect.

In this study, we retrospectively analyzed the treatment response in patients with MG treated with a standardized 3-month protocol with only GCs and compared the criterion based on absolute change of QMGS and percentage of QMGS improvement after the treatment. Due to the skewed distribution of the pre-treatment QMGS in this study, we stratified them into mild, moderate, and severe subgroups to explore the influence of baseline QMGS on the consistency of the criteria.

MATERIALS AND METHODS

Patient Recruitment and Study Design

A total of 257 patients with MG, who had not received any immunological treatments were consecutively enrolled and followed every month till 3 months after treatment, were included in this study. After the pre-treatment QMGS were recorded, GCs equivalent to 0.75 $\sim$ 1 mg/kg/day of prednisone were started. The dosage of GCs was tapered gradually when there was notable improvement, or remained the same as the initial dosage until the end of 3 months. The post-treatment QMGS were recorded. Details of patient recruitment and treatment were expatiated in our previous research (5).

Criterion A was set based on the change of QMGS ($QMGS_{pre-treatment} - QMGS_{post-treatment}$). Improvement $\geq$ 3 points in QMGS or QMGS decreased to 0 after 3-month treatment was defined as responsive to GCs (2, 3). Criterion R was set based on improvement percentage as ($QMGS_{pre-treatment} - QMGS_{post-treatment}$)/$QMGS_{pre-treatment}$. Taking the criterion A as the reference standard, we used receiver operating characteristic (ROC) curve to define the optimum cut-offs for the criterion R in the whole group and three subgroups stratified by pre-treatment QMGS. The consistency was compared between the two criteria in the whole group, as well as in subgroups.

Statistical Analysis

Statistical analyses were performed using IBM SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). The normality of continuous variables was tested by the Kolmogorov–Smirnov test. Continuous variables were presented as mean $\pm$ standard deviation (SD) or median (interquartile range, IQR). Categorical variables were expressed as frequencies (percentages), and the chi-square or Fisher's exact test was used to compare their differences. The optimum cut-offs of criterion R for GC repressiveness were determined by ROC curves (13). Two $\times$ two tables were constructed for GC responsiveness based on relevant cut-offs. Cohen's Kappa test was used to analyze the consistency between the two criteria. Kappa values of 0.21∼0.4 were considered fair, 0.41∼0.60 moderate, 0.61∼0.80 substantial, and 0.81∼1.00 almost perfect (14). A two-tailed $p < 0.05$ was considered significant. Venn diagrams were used to demonstrate the consistent and inconsistent patients by the two criteria, and details of improvement of the inconsistent patients were listed for inspection.

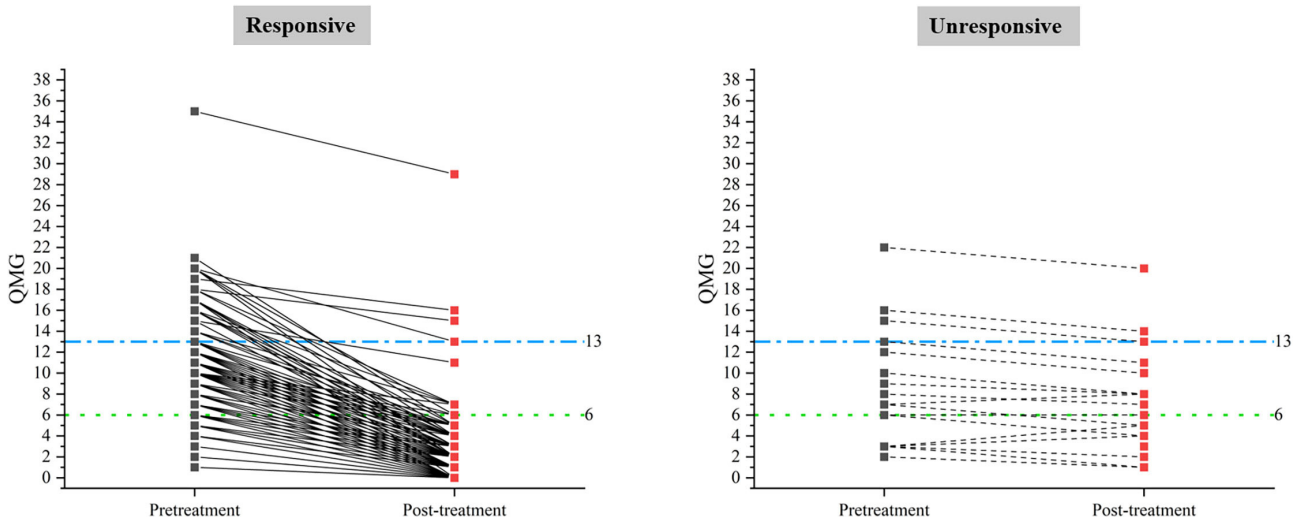


FIGURE 1 | Pre-treatment and post-treatment QMGS in responsive and unresponsive patients classified by criterion A.

RESULTS

General Characteristics

A total of 98 (38.1%) male patients and 159 (61.9%) female patients were included in this study. Onset age ranged from 15 to 80 years old (43.4 ± 16.6). The disease duration prior to treatment ranged from 2 to 48 months (median 4, IQR 2 ∼ 11). The pre-treatment QMGS ranged from 1 to 35 (median 6, IQR 4 ∼ 11). The patients were classified into three subgroups according to baseline QMGS as follows: 105, mild (QMGS 1 ∼ 5); 108, moderate (QMGS 6 ∼ 12); and 44, severe (QMGs ≥ 13) patients. After 3-month GC treatment, the change of QMGS ranged from −2 to 18 (median, 5; IQR, 3 ∼ 8). The demographic and clinical features were summarized in **Supplementary Table 1**, and the changes in absolute QMGS were shown in **Figure 1**.

Responsiveness to GCs

The absolute QMGS changes ranged from −2 to 18 (median, 5; IQR, 3 ∼ 8). The improvement percentages ranged from −66.7 to 100% (median, 86.67%; IQR, 70 ∼ 100%). Based on criterion A, 235 patients (91.44%) were classified as responsive to GCs, and 22 patients (8.56%) as unresponsive. There were significant differences in absolute changes of QMGS ($p < 0.001$) and improvement percentage of QMGS ($p < 0.001$) between responsive and unresponsive groups. There was a significant difference in disease duration before GCs treatment (≤6 months vs. > 6 months, $p = 0.027$) between the two groups. No differences were found in other clinical characteristics between the two groups (**Supplementary Table 1**).

Using the ROC method, an improvement of 51.925% was calculated as the optimum cut-off for criterion R in the whole group. The cut-offs were calculated as 70.835, 36.665, and 15.585% in the mild, moderate, and severe subgroups, respectively (**Supplementary Table 2**).

TABLE 1 | Consistency analysis between Criterion A and Criterion R.

	A+/R+	A+/R-	A-/R-	A-/R+	Kappa value	*P*-value
Criterion R1 (cut-off at 51.925%)						
Total	218	17	20	2	0.639	<0.001
1–5	98	0	5	2	0.824	<0.001
6–12	87	10	11	0	0.639	<0.001
≥13	33	7	4	0	0.462	0.001
Criterion R2 (cut-off at 36.665%)						
Total	226	9	19	3	0.735	<0.001
1–5	98	0	4	3	0.713	<0.001
6–12	93	4	11	0	0.826	<0.001
≥13	35	5	4	0	0.56	0.001

Consistency Between Criterion A and Criterion R

Using the cut-off (51.925%, Criterion R1) derived from all the patients, the Kappa value was 0.639 in the whole group, 0.824 in the mild group, 0.639 in the moderate group, and 0.462 in the severe group (all $p \leq 0.001$, **Table 1**). Because the proportion of patients classified into the moderate group by Criterion A was the largest among the three subgroups, and moderate baseline QMGS was often seen in clinical trials, we used the cut-off derived from these patients (36.665%) to set Criterion R2. With criterion R2, the Kappa values were 0.735, 0.713, 0.826, and 0.56 in the whole group, mild group, moderate group, and the severe group, respectively (all $p \leq 0.001$, **Table 1**). The Kappa values were substantial to almost perfect in the mild and moderate groups and moderate in the severe group between Criterion A and both Criteria R1 and R2.

The Venn diagrams (**Figure 2**) demonstrated that two patients were classified as unresponsive with Criterion A while as

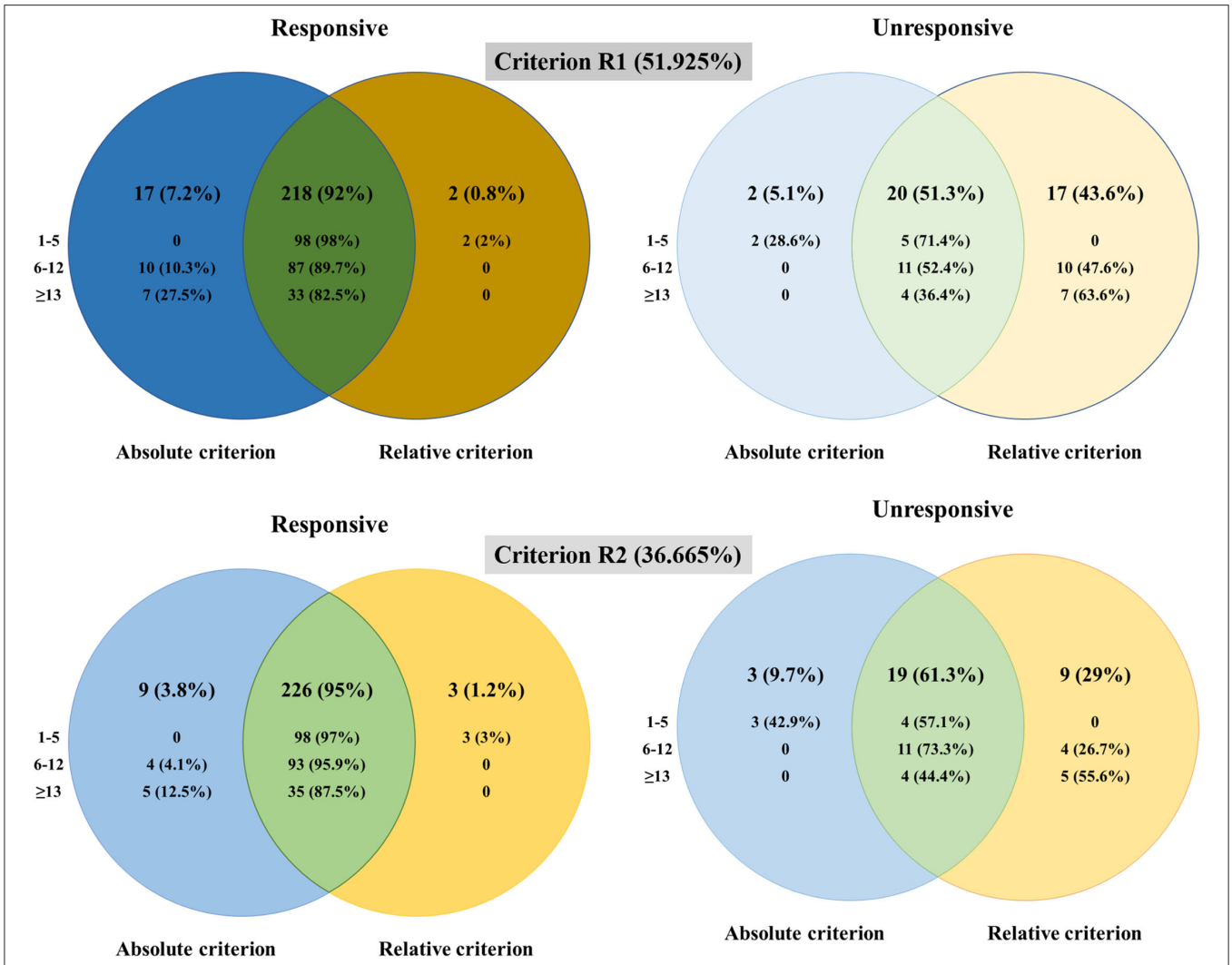


FIGURE 2 | The differences in the patients classified as responsive and unresponsive with different criteria.

responsive with Criterion R1, three patients (including the above two patients) as unresponsive with Criterion A, while as responsive with Criterion R2. This inconsistent pattern was only seen in the mild group. The proportions of patients classified as responsive in the mild group were 98/105 (Criterion A), 100/105 (Criterion R1), and 101/105 (Criterion R2), indicating a strong consistency between Criterion A and Criterion R in the mild group. Even though the changes of QMGS did not reach 3 points, the improvement percentages were 50∼66.7% in these three patients. Seventeen patients were classified as responsive with Criterion A while unresponsive with Criterion R1, and nine patients (included in the above 17 patients) were classified as responsive with Criterion A while unresponsive with Criterion R2. This inconsistent pattern was only seen in the moderate and severe groups. The proportions of the patients classified as unresponsive in the moderate group were 11/108 (Criterion A), 21/108 (Criterion R1), and 15/108 (Criterion R2); unresponsive in the severe group were 4/44 (Criterion A), 11/44 (Criterion R1), and 9/44 (Criterion R2). Even though the change of QMGS reached 3 points, the improvement percentages were 15.79∼50% in the unresponsive patients defined with Criterion R1 and 15.79∼35% in the unresponsive patients defined with Criterion R2 (**Table 2**).

DISCUSSION

A recent study that reported the change in % of normal between original and follow-up visits has shown a strong correlation with the change in QMGS (ΔQMGS) (15), which suggested the potential usage of improvement percentage as the response criterion. In our study, the consistencies were substantial between criteria (A vs. R1 and A vs. R2) in all the patients, substantial to almost perfect in the mild and moderate patients while moderate

TABLE 2 | Clinical features of inconsistent patients in Criterion A and Criterion R.

Criterion			Gender	Onset age	Thymoma	AChRAb	MuSKAb	Onset involvement	Treat in 6 months	QMGS-pre	QMGS-post	Δ QMGS	Δ %
A	R1	R2											
-	-	+	Male	17	-	-	-	Ocular	-	2	1	1	50
-	+	+	Male	68	-	+	-	Ocular	-	3	1	2	66.67
-	+	+	Female	60	-	-	-	Ocular	-	3	1	2	66.67
+	-	-	Male	42	+	+	-	Ocular	-	19	16	3	15.79
+	-	-	Male	34	+	+	-	Generalized	+	18	15	3	16.67
+	-	-	Male	46	+	+	-	Ocular	+	35	29	6	17.14
+	-	-	Female	59	-	+	-	Generalized	+	15	11	4	26.67
+	-	-	Female	46	-	-	-	Generalized	-	10	7	3	30
+	-	-	Male	25	-	+	-	Generalized	+	10	7	3	30
+	-	-	Female	54	-	+	-	Generalized	+	10	7	3	30
+	-	-	Female	20	-	+	-	Generalized	+	10	7	3	30
+	-	-	Female	46	+	+	-	Ocular	+	20	13	7	35
+	-	+	Female	32	+	+	-	Ocular	-	10	6	4	40
+	-	+	Male	75	+	+	-	Generalized	+	10	6	4	40
+	-	+	Female	42	+	+	-	Ocular	+	9	5	4	44.44
+	-	+	Female	72	-	-	+	Ocular	+	6	3	3	50
+	-	+	Female	54	+	+	-	Generalized	-	8	4	4	50
+	-	+	Female	31	-	+	-	Ocular	+	10	5	5	50
+	-	+	Female	36	+	+	-	Ocular	-	14	7	7	50
+	-	+	Female	59	+	+	-	Ocular	-	14	7	7	50

in the severe patients. The Venn diagrams confirmed the inconsistency came from baseline moderate and severe patients.

The two criteria were developed at the group level or the individual level. The confounding role of baseline severity on responsiveness in an individual patient was also noted by Katzberg et al. (4). They proposed using a QMGS cut-off of 2 for patients with a baseline QMGS of < 16 and 3 for those with baseline QMGS > 16. In our study, we used different cut-offs to set Criteria R1 and R2, which resulted in a different consistency. However, the improvement percentages in individual patients were the same whichever the criterion R was used. From the detailed information on inconsistent patients, the diluting effects of baseline severity on responsiveness could be visualized directly. When two patients with the same ΔQMGS of 4 were taken as an example, QMGS decreased from 15 to 11 in one patient, while from 8 to 4 in the other patient. In baseline moderate or severe patients with MG, using the improvement percentage of 36.665% (Criterion R2) as the cutoff of QMGS is closer to our clinical experience.

There were several limitations in our study: First, the pre-treatment QMG score in this study was in skewed distribution; the number of severe patients was much less than the mild and moderate ones. However, skewed data were inevitable in clinical studies. We used the cut-off derived from moderate patients, which constituted the largest proportion of all the patients to overcome this limitation, and acquired substantial consistency between the absolute and relative criteria. However, comparison at the group level could not overcome the bias from skewed distribution in baseline QMGS. The patients who had high baseline scores but smaller Δ QMGS might not have actual improvements, as shown in our study. Second, we lack another reference criterion for which the two criteria could be compared, especially simple patient-reported measures, such as single simple questions (15) or scales, such as MG-ADL or MG-QOL15. Nevertheless, in the short-term evaluation with an interval of 3 months, the slope of the connecting line (pre-treatment QMGS to post-treatment QMGS) in an individual patient might give a clue for the evaluation of the treatment effect. The larger the slope is, the stronger the response is.

CONCLUSION

By determination of the consistency between absolute and relative criteria, this study showed an overall substantial consistency in the short-term treatment response of GC in patients with MG and the inconsistent aspects between the two criteria in subgroups stratified by baseline severity. This will shed light on the definition of responsiveness in both observational studies and clinical trials in MG. The relative criterion should be examined with other quantitative measures of severity to define treatment response in patients with MG.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Beijing Friendship Hospital, Capital Medical University and Ethics Committee of Affiliated Hospital of Qingdao University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

H-FL and Y-XY conceptualized and designed the study and revised the manuscript. H-YL and PJ interpreted the data and wrote the manuscript. H-FL, YX, and Y-XY diagnosed, treated, recruited, and followed up with the patients in this study. H-YL, PJ, and BL performed the statistical analysis. LL and CZ contributed to the discussion. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the National Natural Science Foundation of China (Nos. 81070963, 81771362, and 82171397 to H-FL), the Qingdao Technology Program for Health and Welfare (No. 17-3-3-26-nsh to H-FL), and Research Grant from Qilu Hospital (Qingdao), Cheeloo College of Medicine, Shandong University (QDKY2021RX06 to Y-XY).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.880040/full#supplementary-material

REFERENCES

1. Benatar M, Sanders DB, Burns TM, Cutter GR, Guptill JT, Baggi F,et al. Recommendations for myasthenia gravis clinical trials. *Muscle Nerve.* (2012) 45:909–17. doi: 10.1002/mus.23330
2. Barohn RJ, McIntire D, Herbelin L, Wolfe GI, Nations S, Bryan WW. Reliability testing of the quantitative myasthenia gravis score. *Ann N Y Acad Sci.* (1998) 841:769–72. doi: 10.1111/j.1749-6632.1998.tb11015.x
3. Bedlack RS, Simel DL, Bosworth H, Samsa G, Tucker-Lipscomb B, Sanders DB. Quantitative myasthenia gravis score: assessment of responsiveness and longitudinal validity. *Neurology.* (2005) 64:1968–70. doi: 10.1212/01.WNL.0000163988.28892.79
4. Katzberg HD, Barnett C, Merkies IS, Bril V. Minimal clinically important difference in myasthenia gravis: outcomes from a randomized trial. *Muscle Nerve.* (2014) 49:661–5. doi: 10.1002/mus.23988
5. Xie Y, Meng Y, Li HF, Hong Y, Sun L, Zhu X, et al. GR gene polymorphism is associated with inter-subject variability in response to glucocorticoids in patients with myasthenia gravis. *Eur J Neurol.* (2016) 23:1372–9. doi: 10.1111/ene.13040
6. Li HF, Gao X, Xie YC. Recommendations for myasthenia gravis clinical trials. *Muscle Nerve.* (2013) 47:144–5. doi: 10.1002/mus.23670
7. Wang XY, Xu XH, Sun H, Han X, Zhang H, Guo H. A clinical absolute and relative score system for myasthenia gravis. *Zhonghua Shen Jing Ke Za Zhi.* (1997) 30:87–90
8. Quax RA, Koper JW, Huisman AM, Weel A, Hazes JM, Lamberts SW, et al. Polymorphisms in the glucocorticoid receptor gene and in the glucocorticoid-induced transcript 1 gene are associated with disease activity and response to glucocorticoid bridging therapy in rheumatoid arthritis. *Rheumatol Int.* (2015) 35:1325–33. doi: 10.1007/s00296-015-3235-z
9. Sanders DB, Benatar M, Burns TM, Cutter GR. Reply: to PMID 22581550. *Muscle Nerve.* (2013) 47:145–6. doi: 10.1002/mus.23668
10. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, ET AL. International consensus guidance for management of myasthenia gravis: executive summary. *Neurology.* (2016) 87:419–25. doi: 10.1212/WNL.0000000000002790
11. Verschuuren JJ, Palace J, Murai H, Tannemaat MR, Kaminski HJ, Bril V. Advances and ongoing research in the treatment of autoimmune neuromuscular junction disorders. *Lancet Neurol.* (2022) 21:189–202. doi: 10.1016/S1474-4422(21)00463-4
12. Morren J, Li Y. Maintenance immunosuppression in myasthenia gravis, an update. *J Neurol Sci.* (2020) 410:116648. doi: 10.1016/j.jns.2019.116648
13. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology.* (1982) 143:29–36. doi: 10.1148/radiology.143.1.7063747
14. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics.* (1977) 33:159–74. doi: 10.2307/2529310
15. Abraham A, Breiner A, Barnett C, Katzberg HD, Bril V. The utility of a single simple question in the evaluation of patients with myasthenia gravis. *Muscle Nerve.* (2018) 57:240–4. doi: 10.1002/mus.25720

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Neurology

OPINION
published: 02 June 2022
doi: 10.3389/fneur.2022.839769

User Involvement in Myasthenia Gravis Research

Nils Erik Gilhus[1,2]* *and Sandra Iren Barkås Hovland*[3]

[1] Department of Clinical Medicine, University of Bergen, Bergen, Norway, [2] Department of Neurology, Haukeland University Hospital, Bergen, Norway, [3] Norwegian Association for Muscle and Nerve Disorders, Oslo, Norway

Keywords: myasthenia gravis, user involvement, autoimmunity, patient representation, patient participation

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease with muscle weakness as the main manifestation (1). The disease is chronic, often with life-long symptoms. Most patients need daily immunosuppressive and cholinergic drug treatment (2). Although much is known about disease mechanisms, the cause of MG is unknown, and no curative treatment is available. MG is classified as a rare disease, with an annual incidence 10 per million in most populations, and a prevalence of 150–250 per million (3, 4).

The need for MG research is high. This is true for nearly all aspects of the disease. Genetic and environmental factors interact in causing MG. Most of the genetic predispositions remain unexplained, and we know even less about causative environmental factors. Although effective treatment is available, there are only a few placebo-controlled therapeutic trials. Studies comparing different treatment alternatives are lacking, and prospective, long-term follow-up studies are sparse. Research regarding burden of disease, quality of life, non-muscle symptoms, and the effect of supportive therapies and non-pharmacological interventions has emerged in recent years, but unbiased and well-conducted studies are only a few (5–7).

MG comprises a wide variation of phenotypes. Disease subgroups have been defined from age at symptom debut, generalization of symptoms, autoantibody profile and thymus pathology (8). Combinations of biomarker pattern and clinical manifestations will probably lead to further subgrouping of MG patients in the future and guide a more individually adapted treatment. In addition to phenotypic variation, there are important geographical differences. In China and Japan, there is a group of patients with MG onset in very early childhood (9–11). In Europe and North America, late onset MG is by far the most common type, in part due to demography. Availability of therapeutic and therapeutic alternatives as well as organization of MG care vary even more world-wide.

Patient involvement in health care is not only desirable but is a social, technical, and economic necessity (12). This includes treatment of MG. Patients are generally positive to take part as objects in research projects (13). In addition, they regard their active involvement as user representatives as important. MG research needs the input from patients who have experienced the various symptoms, examinations, and therapies, as well as the multiple consequences of having MG. MG patients know from experience the needs for a precise diagnosis and better treatment, for correct information and more knowledge. The linguistic shift from "patient" to "user" reflects a change in ideology (14). Our recent paper has illustrated the complex needs of MG patients (15). The patients themselves should be partners in the project to improve the present situation. Such user involvement should be adapted according to the phenotypic variation of MG. Some MG research questions are universal, whereas others are specific for children, pregnant women, the very old, immigrants, or other patient subgroups.

OPEN ACCESS

Edited by:
Vera Bril,
University of Toronto, Canada

Reviewed by:
Alessandra Solari,
IRCCS Carlo Besta Neurological Institute Foundation, Italy
Hans Katzberg,
University of Toronto, Canada
Anna Rostedt Punga,
Uppsala University, Sweden
Michael W. Nicolle,
Western University, Canada

***Correspondence:**
Nils Erik Gilhus
nils.gilhus@uib.no

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *20 December 2021*
Accepted: *26 April 2022*
Published: *02 June 2022*

Citation:
Gilhus NE and Hovland SIB (2022) User Involvement in Myasthenia Gravis Research. Front. Neurol. 13:839769. doi: 10.3389/fneur.2022.839769

In this paper, our aim is to examine the relevant literature, make a narrative review, discuss the need of active involvement from MG patients in research projects relevant for this disease, and then conclude with several recommendations applicable in active MG research. User involvement should improve research relevance and quality, but also patient inclusion and continuation rates, project funding, dissemination of the results, and implementation of new knowledge into clinical practice.

PATIENT NEEDS

Most MG patients do well and have a good prognosis (16). Expected life-length is nearly unaffected by the disease in well-developed countries (17, 18). Long-term studies typically report that a clear majority of MG patients are in full or partial remission, with no or only mild symptoms. In contrast, 10–20% of the patients have a disease that is difficult to treat and with a need for intensified treatment, whereas <5% have long-lasting severe MG (10, 16, 19). Many MG patients can function in daily life and partake in the ordinary labor market. However, while MG may be considered less severe than some other neurological and autoimmune conditions, most patients report reduced quality of life.

When questioning MG patients about specific complaints and limitations, it becomes clear that MG is a disease that has an impact on daily life and with a clear need for new and better treatments (5). A broad range of symptoms and deficits can be recorded as scores in MG-specific outcome forms or registered by specific questioning during ordinary consultations (20). In our recent article where we applied the MG patient perspective, we discussed patient needs in detail (15). The article was co-authored by MG experts and user representatives from three different countries.

Burden of MG disease is not clearly related to degree of muscle weakness (21). It depends in part on such phenotypic aspects as sex and age (5). Younger patients and females report more limitations and a poorer disease-specific quality of life. Quality of life does not seem to have improved for MG patients during the last decades, despite more effective immunosuppressive treatment (15). Nearly one third of MG patients answered "no" when asked if they were satisfied with their current MG status (22). In choosing optimal treatment, patients are interested in reports from other patients with the same diagnosis. Such patient experiences can be collected systematically (23).

To the patients it is the overall quality of life that is of greatest concern. Setting realistic expectations of the disease through systematically collected patient data may be beneficial both for them and their families. Patients are disappointed if it turns out that their disease is not as mild and easily managed as they had hoped for. Systematically collected patient experiences are useful not only to other patients but also to researchers and clinicians. Patient organizations typically have programs to get the newly diagnosed in contact with other patients to share experiences and ideas for managing their MG. User representatives could bring such records into research projects.

Muscle strength improvement is the major aim in MG treatment. For the patient, strength in some muscles is crucial, whereas weakness localized to other muscles has less impact. However, improved strength also in muscles not very important for patients' daily life can be crucial for the objective assessment of a new treatment. Fatigue is in some patients a major symptom (6). Patients feel weak and tired, often in most of the body, and this fatigue responds less well to immunosuppressive and anticholinesterase drugs than the muscle weakness. Physician-based and patient-focused assessments are both included in modern MG trials. Side-effects and worries about long-term consequences of the MG therapy are common. This includes infection risk and reduced vaccination response due to immunosuppression (24), but also cancer risk (25). Possible consequences of MG and MG therapy for pregnancy and the developing child mean that many young MG females postpone or abstain from becoming pregnant (26). Pain and depression are more common in MG patients than in controls. Comorbidities are frequent in MG, and especially in elderly patients (25). These comorbidities can be associated with their MG (other autoimmune disorders, thymoma, drug side effects), they can functionally interact with MG (lung disease, orthopedic disorders), or they can just add to the total burden of disease.

MG patients are eager to support research that in the future may improve their function and quality of life. Myasthenia Gravis Foundation of America (MGFA) and similar national MG patient organizations lists research support among their highest priorities (https://myasthenia.org/Research). Most fields of medical research are relevant in the patient perspective and may benefit from user participation. Diagnostic precision is important for both patient and neurologist and should include MG subgroup and phenotype (15). Patients know that research on disease mechanisms are necessary to improve treatment. MG causative factors, both environmental and genetic, might be preventable or possible to modify, and basic research is the way to reveal them. Treatment studies are most important in the patient perspective, not least prospective and controlled studies comparing alternative treatment protocols. MG patients are eager to contribute to research with the aim of defining optimal availability and organization of MG care. Resources and local priorities will influence MG treatment (27). Research regarding best organization should therefore be nationally or regionally adapted, and always with active user participation.

Studies evaluating to what degree diagnostic procedures and treatment of individual MG patients are consistent with generally accepted guidelines and recommendations are much needed (28). Such studies could unveil lack of knowledge, lack of availability and resources, compliance and organizational issues, but also a need for improved cost-benefit considerations in the recommendations. Active study participation from MG patients both in the planning and in the evaluation and dissemination phase should increase the scientific quality and the relevance for clinical practice.

PATIENT INVOLVEMENT

The aim for patient involvement in MG research is to improve quality, increase research output, increase relevance, support dissemination of results, and secure implementation in clinical practice. These are the main reasons why many institutions and funding sources demand user involvement in planned and ongoing medical research. User involvement in research is in addition justified from common ethical ideals. Individuals that are affected by the disease in focus should have the opportunity to influence activities so important for them (14). User involvement ensures that those who are affected can contribute with their knowledge and lived experience. User involvement is well established in most fields of society, including the clinical practice of hospitals and other health institutions. Medical research is such an important sector that broad involvement from the society is necessary. Patients and other users should get the chance to contribute. Their practical participation may be influenced by their MG symptoms such as diplopia (difficult to read) and fatigue.

In the planning phase of a new MG research project, patients can often give important input (29–32). Clinical relevance is an obvious aspect for them to discuss. They may also suggest additional approaches or new topics for research. Furthermore, details regarding recruitment of patients, information to patients, and plans for follow-up may be improved after input from the users. The planning phase often includes applications for project funding. Active user participation will always improve funding possibilities. An increasing number of funding sources demand user involvement.

During an ongoing research project, there will often be less benefit of user involvement. The patients are not researchers and they are not responsible for the daily tasks such as collecting research data. However, they could be involved in questions such as protocol changes, patient participation, or decisions regarding prolongation of an ongoing study.

When all research data have been collected, the results need to be summarized, discussed, and presented. MG patients may have a role in scientific presentations, especially in the interpretation of consequences for diagnosis and treatment, including new or modified recommendations. User representatives should be involved in the dissemination of the research results to the society in general, including patient interest groups and organizations. This should facilitate and speed up implementation of new research results. Patients may help in the wording of the new information and secure the clinical relevance. They may also know and have access to important information channels and patient networks. The researchers are responsible for the scientific communication of the research results. It is equally important to communicate the results to neurologists who treat MG, and to the patients. User representatives are good partners in this process, sometimes also as active presenters to an audience.

Patient representation can be secured through surveys, but better through direct involvement, sometimes even as coauthors. However, user representative and co-researcher usually represent two different roles. Patient representatives are often required to get funding, and not all of them are truly involved in the research. To get the full value of the representatives, they need to be properly involved. They must be both able and willing to contribute to the project.

Guideline documents for MG treatment and diagnosis are widely read and cited, and their recommendations are usually accepted and implemented. We recommend always to involve MG patients in the work on such documents. Their involvement, especially in the discussion and writing stages, should promote a broad evaluation of all relevant factors before reaching a decision. Users may suggest and support inclusion of additional items for evaluation, for example regarding physical training, diet, sleep, pain control, long-term side-effects, and quality of life.

User involvement increases the chances for research funding. Funding institutions that demand such involvement grade the patient involvement in the same way as other aspects of the application. Our experience is that a standard statement from a MG patient organization confirming their willingness to cooperate and be involved in the planned project has become standard practice. More rarely we see that the users have been involved already and with specification of their input. Good practice implicates that they have contributed to the application and the project plan. The user representatives should be named, and their planned contribution should be described in the same way as for other partners in the project. It is wise to state where the user input will increase quality and relevance, but also where users will not have an active role.

How the user representatives are included in the MG research group may vary. The involvement should depend on interests and qualifications of the representatives, and on the research questions of the group (33). Usually, it is not meaningful for either patients or researchers that they take part in all meetings and in the day-to-day work. However, regular contact is important to secure influence, mutual interest, and interaction (14). Providing information, support and feedback to the user representatives is a key to effective engagement. In selected articles, the user representatives may appear as coauthors as they have contributed in accordance with the Vancouver requirements and have responsibility for the full content of the final article. Typical examples could be guideline documents and policy papers (2). For most articles, a formal acknowledgment of their contribution is appropriate.

Some research groups offer an honorarium to their user representatives. This formalizes the cooperation and secures involvement. It puts this research partner in a special position compared to the rest of the participants, but it may hamper a more informal and flexible cooperation. For some MG patients, such payment represents a token of appreciation and boosts further involvement. Expenses as a user representative or any loss of ordinary income should usually be compensated.

User representatives combine several positions (34). They contribute as co-researchers with direct advice. They use their individual MG experience. They represent their patient organization and network, sometimes including their experiences as representatives in previous research projects. Their ordinary professional education and work comes into play. Finally, they may take the position of the concerned citizen, for example regarding health priorities, gender issues, and ethical aspects.

Guidelines have been developed to govern user involvement in research (12, 34, 35). However, challenges persist. They include lack of support and respect, imbalance of power, and lack of acknowledgment of the patients' true experience. Deviations from the agreed principles in the ongoing work are not uncommon, like other research collaborations. A pitfall to avoid is that user involvement takes too much time and resources, even leading to a reduction in research quality and quantity (36). Frameworks and tools have been suggested to facilitate user involvement as a partnership (12, 34, 35). We discuss most of these tools in this article.

CHOOSING PATIENT REPRESENTATIVES

For MG research projects, patients with an experience of MG and the consequences of muscle weakness should be chosen. MG challenges are specific and complex. The value of user involvement relies on self-experience of MG symptoms and MG impairment. Patients with other disorders, for example muscle or nerve disorders, will not be able to give this specific input. There may be a temptation to recruit patients who are at the same time health professionals. We will advise against such practice as it may blur the patient and outside perspective. On the other hand, higher education and professional experience may lead to a broader participation and a hybrid position of both lay and professional expertise on research, further strengthening the collaboration (31). A patient representative who is not a healthcare professional may bring something new to the project and even help uncover confirmation bias. Such representatives may bring to light new aspects and see the project from an alternative angle. Both researcher and user representative need to reflect on their position in the partnership (37).

MG phenotype varies. It is usually not possible to include both a youngster and an elderly person, one with a mild disease and one who have experienced an MG crisis, or user representatives from all defined MG-subgroups. One or two patients need to cover all aspects. However, for a research group with a special interest in MG crisis, they should involve a patient who have experienced this manifestation. For our research group with an interest in pregnancy and consequences for the child, we have included a young female with children. This ensures the relevance of the patient perspective. For juvenile MG, the perspective of the parents is highly relevant, and guardians can be chosen as user representatives in some projects.

MG patient representatives may be recruited directly by the research group from their patient population. A good alternative is to ask the local or national MG patient organization to find a motivated and able candidate. This should strengthen the responsibilities of all partners and secure interaction with the wider patient community. Some hospitals have user panels that are willing to assist in finding representatives to research projects. However, such representatives should be true MG patients, not just professionals working in an interest organization.

MG patient representatives in a research project may sometimes feel lonely among the group of professional researchers (32). Input from other patients and other user representatives benefits their contribution and increase their motivation. Such input can most importantly come from MG patient networks and organizations, but also from networks of patient representatives for various other neurological and non-neurological disorders.

User representatives will be resourceful, interested in research, and usually well adapted in society. The same is usually true for the active MG researchers. In contrast, MG patients with the highest needs are often those with the least resources; poor socioeconomic conditions, lack of near family and friends, comorbidities, sometimes abuse. Such patients may be disengaged from the medical system. They are not good candidates as user representatives in research projects as they will be unable to contribute properly. However, it is important that the perspective that they represent is included both in the planning and execution of the project, and in the dissemination and implementation of the results.

RECOMMENDATIONS

All MG research groups should have formal cooperation with user representatives that give regular input to each project. These representatives should be patients who have MG. The focus for the research group should have a strong influence on the choice of user representative. This representative should be involved in the discussions of all relevant questions during the research process. The MG user contribution is especially important in the planning phase of the project, in recruiting MG patients to the project, in the dissemination of results, and for the implementation of the new findings into clinical practice. In applications for research funding, patient representatives should be involved early, and their contribution throughout the project should be specified. Partnership between patients and MG researchers increases research quality and relevance, is motivating for the researchers, and secures support from the society.

AUTHOR CONTRIBUTIONS

NG conceived the idea and designed the study, searched the literature, and wrote the first draft of the manuscript. SH contributed in planning of the study, searched the literature, and wrote parts of the manuscript. Both authors contributed to the article and approved the submitted version.

REFERENCES

1. Gilhus NE. Myasthenia gravis. *New Engl J Med.* (2016) 375:2570–81. doi: 10.1056/NEJMra1602678

2. Skeie GO, Apostolski S, Evoli A, Gilhus NE, Illa I, Harms L, et al. Guidelines for treatment of autoimmune neuromuscular transmission disorders. *Eur J Neurol.* (2010) 17:893–902. doi: 10.1111/j.1468-1331.2010.03019.x

3. Gilhus NE, Tzartos S, Evoli A, Palaces J, Burns TM, Verschuuren J. Myasthenia gravis. *Nat Rev Dis Prim.* (2019) 5:30. doi: 10.1038/s41572-019-0079-y
4. Chen JS, Tian DC, Zhang C, Li ZX, Zhai Y, Xiu YW, et al. Incidence, mortality, and economic burden of myasthenia gravis in China: a nationwide population-based study. *Lancet Regional Health Western Pacific.* (2020) 5:100063. doi: 10.1016/j.lanwpc.2020.100063
5. Boldingh MI, Dekker L, Maniaol AH, Brunborg C, Lipka AF, Niks EH, et al. An up-date on health-related quality of life in myasthenia gravis - results from population based cohorts. *Health Qual Life Outcomes.* (2015) 13:1–4. doi: 10.1186/s12955-015-0298-1
6. Ruiter AM, Verschuuren J, Tannemaat MR. Fatigue in patients with myasthenia gravis. A systematic review of the literature. *Neuromuscular Disord.* (2020) 30:631–39. doi: 10.1016/j.nmd.2020.06.010
7. Dong D, Chong MKC, Wu YS, Kaminski H, Cutter G, Xu XH, et al. Gender differences in quality of life among patients with myasthenia gravis in China. *Health Qual Life Outcomes.* (2020) 18: doi: 10.1186/s12955-020-01549-z
8. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol.* (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3
9. Hong Y, Skeie GO, Zisimopoulou P, Karagiorgou K, Tzartos SJ, Gao X, et al. Juvenile-onset myasthenia gravis: autoantibody status, clinical characteristics and genetic polymorphisms. *J Neurol.* (2017) 264:955–62. doi: 10.1007/s00415-017-8478-z
10. Huang X, Liu WB, Men LN, Feng HY, Li Y, Luo CM, et al. Clinical features of myasthenia gravis in southern China: a retrospective review of 2,154 cases over 22 years. *Neurol Sci.* (2013) 34:911–7. doi: 10.1007/s10072-012-1157-z
11. Gui MC, Luo X, Lin J, Li Y, Zhang M, Zhang XF, et al. Long-term outcome of 424 childhood-onset myasthenia gravis patients. *J Neurol.* (2015) 262:823–30. doi: 10.1007/s00415-015-7638-2
12. Baines RL, de Bere SR. Optimizing patient and public involvement (PPI): identifying its "essential" and "desirable" principles using a systematic review and modified Delphi methodology. *Health Expect.* (2018) 21:327–35. doi: 10.1111/hex.12618
13. Purdy S, Finkelstein JA, Fletcher R, Christiansen C, Inui TS. Patient participation in research in the managed care environment: key perceptions of members in an HMO. *J Gen Intern Med.* (2000) 15:492–5. doi: 10.1046/j.1525-1497.2000.07025.x
14. Stuhlfauth S, Knutsen IR, Foss IC. Guidelines as governance: critical reflections from a documentary analysis of guidelines to support user involvement in research. *Nurs Inq.* (2021) 28:e12378. doi: 10.1111/nin.12378
15. Gilhus NE, Verschuuren J, Hovland SIB, Simmonds H, Groot F, Palace J. Myasthenia gravis: do not forget the patient perspective. *Neuromuscular Disord.* (2021) 31:1287–95. doi: 10.1016/j.nmd.2021.07.396
16. Andersen JB, Owe JF, Engeland A, Gilhus NE. Total drug treatment and comorbidity in myasthenia gravis: a population-based cohort study. *Eur J Neurol.* (2014) 21:948–55. doi: 10.1111/ene.12439
17. Owe JF, Daltveit AK, Gilhus NE. Causes of death among patients with myasthenia gravis in Norway between 1951 and 2001. *J Neurol Neurosurg Psychiatry.* (2006) 77:203–7. doi: 10.1136/jnnp.2005.072355
18. Hansen JS, Danielsen DH, Somnier FE, Froslev T, Jakobsen J, Johnsen SP, et al. Mortality in myasthenia gravis: a nationwide population-based follow-up study in Denmark. *Muscle Nerve.* (2016) 53:73–7. doi: 10.1002/mus.24697
19. Mantegazza R, Antozzi C. When myasthenia gravis is deemed refractory: clinical signposts and treatment strategies. *Ther Adv Neurol Disord.* (2018) 11:1–11. doi: 10.1177/1756285617749134
20. Barnett C, Bril V, Kapral M, Kulkarni A, Davis AM. Development and validation of the myasthenia gravis impairment index. *Neurology.* (2016) 87:879–86. doi: 10.1212/WNL.0000000000002971
21. Cutter G, Xin HC, Aban I, Burns TM, Allman PH, Farzaneh-Far R, et al. Cross-sectional analysis of the myasthenia gravis patient registry: disability and treatment. *Muscle Nerve.* (2019) 60:707–15. doi: 10.1002/mus.26695
22. Menon D, Barnett C, Bril V. Comparison of the single simple question and the patient acceptable symptom state in myasthenia gravis. *Eur J Neurol.* (2020) 27:2286–91 doi: 10.1111/ene.14397
23. Barabasch A, Riemann-Lorenz K, Kofahl C, Scheiderbauer J, Eklund D, Kleiter I, et al. Impact of a multimedia website with patient experiences of multiple sclerosis (PExMS) on immunotherapy decision-making: study protocol for a pilot randomised controlled trial in a mixed-methods design. *Pilot Feasibility Stud.* (2021) 7:16 doi: 10.1186/s40814-020-00749-0
24. Gilhus NE, Romi F, Hong Y, Skeie GO. Myasthenia gravis and infectious disease. *J Neurol.* (2018) 265:1251–8. doi: 10.1007/s00415-018-8751-9
25. Gilhus NE, Nacu A, Andersen JB, Owe JF. Myasthenia gravis and risks for comorbidity. *Eur J Neurol.* (2015) 22:17–23. doi: 10.1111/ene.12599
26. Ohlraun S, Hoffmann S, Klehmet J, Kohler S, Grittner U, Schneider A, et al. Impact of myasthenia gravis on family planning: how do women with myasthenia gravis decide and why? *Muscle Nerve.* (2015) 52:371–9. doi: 10.1002/mus.24556
27. Recasens BB, Rubio MA. Neuromuscular diseases care in the era of COVID-19. *Front Neurol.* (2020) 11:588929. doi: 10.3389/fneur.2020.588929
28. Narayanaswami P, Sanders DB, Wolfe G, Benatar M, Cea G, Evoli A, et al. International consensus guidance for management of myasthenia gravis 2020 update. *Neurology.* (2021) 96:114–22. doi: 10.1212/WNL.0000000000011124
29. Bird M, Ouellette C, Whitmore C, Li L, Nair K, McGillion MH, et al. Preparing for patient partnership: a scoping review of patient partner engagement and evaluation in research. *Health Expect.* (2020) 23:523–39. doi: 10.1111/hex.13040
30. Brett J, Staniszewska S, Mockford C, Herron-Marx S, Hughes J, Tysall C, et al. Mapping the impact of patient and public involvement on health and social care research: a systematic review. *Health Expect.* (2014) 17:637–50. doi: 10.1111/j.1369-7625.2012.00795.x
31. Domecq JP, Prutsky G, Elraiyah T, Wang Z, Nabhan M, Shippee N, et al. Patient engagement in research: a systematic review. *BMC Health Serv Res.* (2014) 14:89. doi: 10.1186/1472-6963-14-89
32. Thompson J, Barber R, Ward PR, Boote JD, Cooper CL, Armitage CJ, et al. Health researchers' attitudes towards public involvement in health research. *Health Expect.* (2009) 12:209–220. doi: 10.1111/j.1369-7625.2009.00532.x
33. Iwarsson S, Edberg AK, Ivanoff SD, Hanson E, Jonson H, Schmidt S. Understanding user involvement in research in aging and health. *Gerontol Geriatr Med.* (2019) 5:2333721419897781. doi: 10.1177/2333721419897781
34. Solvang PK, Sveen U, Soberg HL. User involvement in the making: positions and types of knowledge enacted in the interaction between service users and researchers in user panel meetings. *Health Expect.* (2021) 24:1424–1432. doi: 10.1111/hex.13281
35. Greenhalgh T, Hinton L, Finlay T, Macfarlane A, Fahy N, Clyde B, et al. Frameworks for supporting patient and public involvement in research: systematic review and co-design pilot. *Health Expect.* (2019) 22:785–801. doi: 10.1111/hex.12888
36. Malterud K, Elvbakken KT. Patients participating as co-researchers in health research: A systematic review of outcomes and experiences. *Scand J Public Health.* (2020) 48:617–28. doi: 10.1177/1403494819863514
37. Maslin-Prothero S. Developing user involvement in research. *J Clin Nurs.* (2003) 12:412–21. doi: 10.1046/j.1365-2702.2003.00755.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 13 June 2022
doi: 10.3389/fneur.2022.877895

Long-Term Efficacy of Non-steroid Immunosuppressive Agents in Anti-Muscle-Specific Kinase Positive Myasthenia Gravis Patients: A Prospective Study

Ying Tan[1], *Jiayu Shi*[1], *Yangyu Huang*[1], *Ke Li*[1], *Jingwen Yan*[1], *Li Zhu*[2], *Yuzhou Guan*[1]* *and Liying Cui*[1]

[1] *Department of Neurology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China,* [2] *Department of Nuclear Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Science, Beijing, China*

OPEN ACCESS

Edited by:
Hai-Feng Li,
Capital Medical University, China

Reviewed by:
Vincenzo Di Stefano,
University of Palermo, Italy
Domizia Vecchio,
University of Eastern Piedmont, Italy

***Correspondence:**
Yuzhou Guan
guanyz001@163.com

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: *17 February 2022*
Accepted: *25 April 2022*
Published: *13 June 2022*

Citation:
Tan Y, Shi J, Huang Y, Li K, Yan J, Zhu L, Guan Y and Cui L (2022) Long-Term Efficacy of Non-steroid Immunosuppressive Agents in Anti-Muscle-Specific Kinase Positive Myasthenia Gravis Patients: A Prospective Study. Front. Neurol. 13:877895. doi: 10.3389/fneur.2022.877895

Background and Purpose: Anti-muscle-specific kinase (MuSK) positive myasthenia gravis (MG) is characterized by a high relapsing rate, thus, choosing the appropriate oral drug regimen is a challenge. This study aimed to evaluate the efficacy of oral immunosuppressants (IS) in preventing relapse in MuSK-MG.

Methods: This prospective cohort observational study included patients with MuSK-MG at Peking Union Medical College Hospital between January 1, 2018, and November 15, 2021. The patients were divided into 2 groups: those with (IS+) or without (IS-) non-steroid immunosuppressive agents. The primary outcome was relapsed at follow-up, and the log-rank test was used to compare the proportion of maintenance-free relapse between the groups; hazard ratio (HR) was calculated using the Cox proportional hazards models.

Results: Fifty-three of 59 patients with MuSK-MG were included in the cohort, 14 were in the IS+ group, and 39 were in the IS- group. Twenty-four cases in the cohort experienced relapse at least once; the relapse rate was 2/14 (14.3%) in the IS+ group and 22/39 (56.4%) in the IS- group. At the end of follow-up, the proportion of maintenance-free relapse was significantly different between the two groups (log-rank $\chi^2 = 4.94$, $P = 0.02$). Of all the potential confounders, only the use of IS was associated with a reduced risk of relapse. The HR for relapse among patients in the IS+ group was 0.21 (95%CI 0.05–0.58) and was 0.23 (95%CI 0.05–0.93) in a model adjusted for age, sex, relapse history, highest Myasthenia Gravis Foundation of America (MGFA), and accumulated time of steroid therapy.

Conclusions: This study provides evidence that oral non-steroid immunosuppressive agents may be beneficial in reducing relapse in patients with MuSK-MG.

Keywords: myasthenia gravis, minimal manifestation status, autoimmune, anti-AChR antibody, anti-MuSK antibody

INTRODUCTION

Myasthenia gravis (MG) is caused by antibodies directed against the acetylcholine receptor (AChR), or other structural proteins of the neuromuscular junction. In 2001, 70% of AchR-Ab-seronegative MG patients were discovered positive in antibodies against muscle-specific kinase (MuSK) (1). The activation of MuSK, anchored in skeletal muscle, is responsible for the clustering of AChR at the neuromuscular junction (2). Patients with MuSK antibody-positive MG often have facial, neck, and respiratory weakness, but they have less prominent ocular findings compared with AchR antibody-positive MG.

The anti-MuSK subtype of MG presents a different response to immunomodulatory regimens compared to AchR MG, the proportion of patients with MuSK-MG requiring high doses and prolonged treatment to achieve full control of the disease seems to be higher (3–6). In most instances, patients with MuSK-MG respond to immunosuppressants (IS). Steroid, azathioprine (AZA), tacrolimus (TAC), mycophenolate mofetil (MMF), cyclosporine A (CsA), methotrexate (MTX), and rituximab (RTX) have been tried with success in patients with MuSK-MG and patients with AchR-MG (7–14). However, there is a lack of prospective data and a large sample to verify the effect of using oral non-steroid IS in MuSK-MG. Furthermore, previous studies did not consider exacerbation as a primary endpoint, and information about relapse is lacking (15, 16). Here, we conducted a prospective observational cohort study in Chinese patients with MuSK antibody-positive MG to determine the association between relapse risk and the use of oral non-steroid IS.

PATIENTS AND METHODS

Participants

Patients were identified through the Peking Union Medical College Hospital (PUMCH) MG registry platform. The study was approved by the regional ethics committee of PUMCH, and participants provided written consent to registration in the MG registry and the use of recorded data for research purposes. The study was conducted from January 1, 2016 to November 30, 2021. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies.

All patients included were diagnosed with MG. We prospectively collected demographic information and data on the course of illness, medication, neurological physical examinations, MGFA classification, MGC, MG-ADL scores, and Repetitive Nerve Stimulation (RNS) results. Patients' serum samples were acquired prospectively at enrolment. Serum AChR antibody titers and MuSK antibody were estimated by the immunoprecipitation methods using ^{125}I-alpha-bungarotoxin and ^{125}I-MuSK, respectively (RSR Limited, Cardiff, UK). All MuSK antibody-positive patients with MG were enrolled in the cohort.

Therapeutic Regimens

In this study, participating patients with MuSK-MG, either received or did not receive IS after a complete discussion with the neurologists. Financial burden and potential AEs are the main concerns for patients who refused IS. We defined the IS+ group as treatment with one or more non-steroid IS for at least 6 months and the IS- group as treatment with only steroids during the follow-up. The following exclusion criteria were applied: the duration of observation was $<$ 6 months, $<$ 2 follow-up visits, concurrent neurologic diseases interfering with assessment, and immunosuppressive therapy for other indications during the observation.

Non-steroid IS dosing range was as follows: AZA 100–150 mg/day, TAC 3–5 mg/day, and MMF 1,000–3,000 mg/day. In the long-term follow-up, specialists set the achievement of MMS and better as the treatment goal and reduced the steroids to the minimum maintenance dose according to the long-term side effects of the steroids. All IS+ and IS- patients were treated with rescue therapy after an exacerbation, including intravenous methylprednisolone pulse therapy, intravenous immunoglobin injections (IVIG), and plasma exchange (PLEX).

Follow-Up and Outcome Measurements

Patients were followed up by the specialist group every 6 month. Medication, MGC score, MG-ADL score, MG-PIS classification, and MuSK antibody results were recorded at each follow-up visit. Drug side effects were regularly monitored.

The study's primary outcome was relapsed, defined as a physician-confirmed exacerbation of MG in a previously stable state, except for other possible contributors to the exacerbation of weakness, such as electrolyte disturbance, infection, etc. A general ΔMGC score of $>$ or equal to 3, treatment with rescue therapy, and/or hospitalization was considered clinically significant (17). The interval between the first relapse and time 0 after enrolment was recorded in days. We also assessed whether the association between IS therapy and relapse differed between the following subgroups: age at disease onset, high *vs.* low MuSK antibody titer, onset type, high *vs.* low MGFA subtype, and with *vs.* without relapse history before enrollment.

Sample Size Calculation

The primary endpoint of this study was to calculate whether the use of IS significantly reduced the risk of relapse, and the results were calculated according to Cox risk proportional model, with Power calculated based on reference to Rosner and Freedman et al. The relapse rate of MuSK-MG in the IS+ group was 15% according to Evoli et al. (6) and 56% according to Guptill et al. (15), in the IS- group treated with steroids alone. Assuming IS+ and IS- patients were divided in a 1:1 ratio, at an expected dropout rate of 5%, 26 patients were required in each group. Then, we further assumed a postulated hazard ratio (HR) of 0.25 and α (two-sided) of 0.05, this study had more than 80% power to detect an HR of 0.25 or lower.

Statistical Analysis

Continuous variables that were not normally distributed were expressed as the median and interquartile range (median, IQR)

and categorical variables were expressed as frequencies and percentages (%). The χ^2 and Mann-Whitney U tests were used for the comparison of categorical and continuous variables that were not normally distributed between the IS+ and IS- groups, respectively. Group differences in relapse risk were assessed using Kaplan-Meier curves, while univariate and multivariate

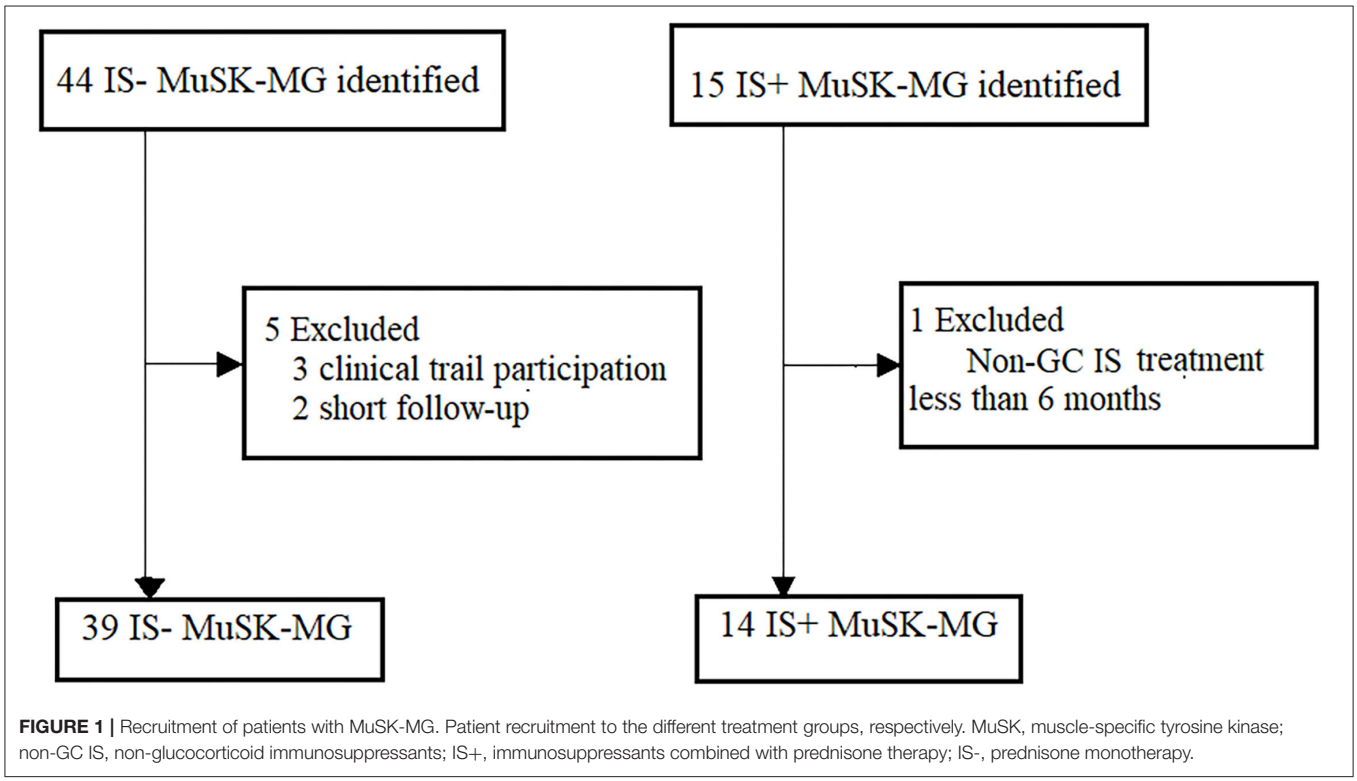


FIGURE 1 | Recruitment of patients with MuSK-MG. Patient recruitment to the different treatment groups, respectively. MuSK, muscle-specific tyrosine kinase; non-GC IS, non-glucocorticoid immunosuppressants; IS+, immunosuppressants combined with prednisone therapy; IS-, prednisone monotherapy.

TABLE 1A | Baseline characteristics of MuSK-MG.

Name	Results			
	Total	IS+ group	IS- group	*P*-value
Number of patients, *n*/total (%)	53/53 (100%)	14/53(26.42%)	39/53 (73.58%)	NA
Onset age[c], y	47 (34, 55)	46.50 (28.50, 52.25)	44 (34, 53)	0.96[a]
Late onset (≥50), *n*/total (%)	20/53 (37.74%)	6/14 (42.86%)	13/39 (33.33%)	0.54[b]
Female, *n*/total, (%)	39/53 (73.58%)	10/14 (71.43%)	29/39 (74.36%)	0.53[b]
Information prior to time 0				
First onset muscles, *n*/total (%)				0.75[b]
Extraocular muscle	35/53	10/14	25/39	
Bulbar muscle or neck or facial muscle	12/53	2/14	10/39	
Limb or trunk muscle	6/53	2/14	4/39	
Disease duration, months	12 (6, 47)	35 (11.8, 69)	12 (4, 24)	0.05[a]
History of relapse, *n*/total (%)	16/53 (30.19%)	11/14 (78.57%)	5/39 (12.92%)	<0.01[b]
Steroid therapy, *n*/total (%)	24/53 (45.28)	10/14 (71.43%)	14/39 (35.90%)	0.03[b]
History of IS therapy, *n*/total (%)	6/53 (11.32%)	4/14 (28.57%)	2/39 (5.13%)	0.04[b]
IVIG within 3 months, *n*/total	22/53	5/14	17/39	0.74[b]
Information at time 0				
MGC score[c]	6 (3, 12)	4 (0, 12.75)	6 (3, 12)	0.42[a]
ADL score[c]	3 (2, 8)	2.5 (0, 9.5)	5 (2, 8)	0.39[a]
Muscle atrophy, *n*/total	6/53	3/14	3/39	0.21[b]
limb/facial muscle/lingual muscle, *n*	4/1/1	2/1/0	2/0/1	
MuSK-Ab[c]	1.13 (0.82, 1.45)	1.36 (0.83, 1.46)	1.07 (0.81, 1.43)	0.36[a]
RNS decrement, *n*/total (%)	38/51(74.5%)	11/14 (78.6%)	27/37(73.0%)	1.00[b]

[a]*Mann-Whitney U test;* [b]*Fisher exact test;* [c]*Described by Median (IQR).*

hazard ratios (HRs) were assessed from Cox proportional hazards regression. The association of IS application with relapse of MG was compared in each subgroup: gender, age of onset, first onset muscle group, highest MGFA, relapse before time 0, initial MuSK antibody titters, and different disease duration subgroups. Stratified Cox risk proportional regression models were applied for subgroup analysis. All statistical results were significant by taking an $\alpha < 0.05$, two-sided test. Prism 7 (GraphPad Software) was used for unadjusted statistical tests. SPSS 28.0 (IBM) was used for statistical analysis.

RESULTS

Patient Characteristics

The study flowchart is shown in **Figure 1**. We identified 59 patients with MuSK antibody-positive in the database, 53 patients fulfilled the inclusion criteria and exclusion criteria (**Figure 1**). Of the 53 eligible patients, 39 patients (73.60%) were women; the median age of onset was 47 years (IQR 34–55). Fourteen of them received IS and steroid combination therapy [10 (71.40%) women]. The baseline characteristics of the two groups were largely comparable, including age at onset, gender composition,

TABLE 1B | Follow up outcomes of MuSK-MG.

Name	Results			*P*-value
	Total	IS+ group	IS- group	
Information after enrollment				
Highest MGFA classification, *n* (*n*/total)				0.65[b]
I	1 (1/53)	0	1 (1/39)	
II	30 (30/53)	7 (7/14)	23 (23/39)	
IIa/IIb, *n*	0/28	0/7	0/7	
III	10 (10/53)	4 (4/14)	6 (6/39)	
IIIa/IIIb, *n*	0/8	0/4	0/6	
IV	5 (5/53)	2 (2/14)	3 (3/39)	
IVa/IVb, *n*	0/4	0/2	0/1	
V	7 (7/53)	1 (1/14)	6 (6/39)	
Relapse, *n*/total (%)				0.01[b]
0	29/53 (54.72%)	12/14 (85.71%)	17/39 (43.59%)	
≥1	24/53 (45.28%)	2/14 (14.29%)	22/39 (56.41%)	
Gap from time0 to first relapse [d], days	0 (0, 420)	NA[c]	210 (0, 480)	NA
Final visit				
MGC score[d]	0 (0, 3)	0 (0, 3.50)	0 (0, 3)	0.63[a]
ADL score[d]	0 (0, 2)	0 (0, 2)	0 (0, 2)	0.56[a]
PIS classification, *n*/total (%)				0.66[b]
MM or better	30/53 (56.60%)	9/14 (64.29%)	21/39 (53.85%)	
Improved	17/53 (32.08%)	5/14 (35.71%)	12/39 (30.77%)	
Unchanged	1/53 (1.89%)	0	1/39 (2.56%)	
Worse	4/53 (7.55%)	0	4/39 (10.26%)	
Died	1/53 (1.89%)	0	1/39 (2.56%)	
Follow up time[d], days	814 (540, 1110)	780 (352, 915)	840(630, 1290)	0.79[a]
Improving MGC[d]	3 (0, 9)	3 (0, 5.3)	3 (0, 11)	0.70[a]
Improving ADL[d]	2 (0, 5)	2 (0, 5.5)	2 (0, 6)	0.81[a]
Rate of steroid use, *n*/total (%)	50/53 (94.34%)	14/14 (100%)	36/39 (92.31%)	0.54[b]
Highest steroid dose, mg/d, *n*/total				0.02[b]
0	3/53	0	3/39	
1–20	0	0	0	
20–50	28/53	4/14	24/39	
>51	22/53	10/14	12/39	
Number of stop using steroid, *n*/total	8/53	0	8/39	0.09[b]
Steroid dose at last visit[d], mg/d	10 (5,13.80)	10 (9.38, 15)	10 (5, 12)	0.41[a]
Accumulated time for steroid[d], days	1020 (408.5, 1545)	1020 (414, 1507)	720 (390, 1470)	<0.01[a]

[a]*Mann-Whitney U test;* [b]*Fisher exact test;* [c]*Uncountable because of only two cases with relapsing events in IS+ group, 1 was at 570 days and 1 was at 1,080 days after enrollment;* [d]*Described by Median (IQR).*

first muscle group affected, highest MGFA, severity as reflected by the most recent MGC/ADL score before time 0, MuSK antibody titers at time 0, RNS results, number of cases with muscle atrophy, or duration of disease before time 0 (**Table 1A**).

Eleven (78.6%) and 5 (12.8%) patients had a history of MG relapse before enrolment in the two groups, with a statistically significant difference ($p < 0.01$). Six patients had a history of non-steroid IS therapy before enrolment, four in the IS+ group and two in the IS- group, two patients discontinued AZA treatment due to adverse events (AEs) before enrolment: 1 with drug-related granulocyte deficiency and another with an allergic reaction at the first week. The number of patients accepting steroid therapy at time 0 was 10/14 (71.4%) in the IS+ group and 14/39 (35.9%) in the IS- group, with statistically significant difference. The number of patients with a history of IVIG application within the 3 months prior to time 0 was 5 (35.7%) and 17 (43.6%) in the IS+ group and the IS- group, with no statistical difference ($p = 0.74$). One patient had undergone thymectomy, and the interval from surgery to enrolment was up to 4 years.

Follow-Up Outcomes

The number of patients receiving steroids in the two groups was 14/14 cases (100%) and 36/39 cases (92.3%), respectively. The median daily dosage of steroids was comparable between the IS+ group and the IS- group at the end of follow-up (10 [9.38–15] mg and 10 [5–12], $p = 0.40$). The number of patients who successfully discontinued steroids was slightly greater in the IS- group (3/39) than that in the IS+ group (0/14), although the results were not statistically different ($p = 0.09$). The other three outcomes, including ADL score, MGC score, improving in ADL, and improvement in QMG, did not have statistical differences in the IS+ group and IS- group (**Table 1B**).

Non-steroid IS medications included AZA (n = 9, 9/14, 48%) and TAC (n = 5, 5/14, 35.7%). No participants discontinued IS therapy owing to severe AEs during follow-up, thus, suggesting good tolerability. None of the patients was maintained on ChE-I at the end of the follow-up.

Effects of Intervention

The median duration of observation was comparable between the IS+ group and the IS- group (780 [352, 915] days vs. 840 [630, 1290] days, $p = 0.70$). Log-rank tests did not reveal an association between relapse and factors, such as gender, initial symptoms, history of previous relapses, duration of disease before time 0, antibody titer at time 0, highest MGFA classification, and length of steroid use (**Table 2**).

The IS was also associated with a longer duration of remission than steroid monotherapy (median 210 [0–480] days for steroid monotherapy; data not available for the IS+ group since 12/14 patients remained stable; HR = 0.21, 95% CI,0.05–0.58, $p = 0.03$, **Figure 2**). This association remained statistically significant after adjustment for age of onset, gender, relapse history, highest MGFA, and accumulation of steroid application (HR = 0.23, 95% CI,0.05–0.93, $p = 0.04$: **Table 3**). The proportion of patients in a clinically stable state at 500 and 1000 days was higher with IS than with steroid monotherapy (500 days: 14/14 [100%] IS treatment *vs.* 25/39 [64.1%] controls, $p < 0.01$; 1000 days: 13/14 [92.9%] and 15/39 [38.5%], $p < 0.01$).

TABLE 2 | Log-Rank test of relapse and demographic characteristics.

Name	Relapse			
	Median survival time	95%CI		Log-Rank *p*-value
Type of sex				0.61
Male	1,080	306.71	1853.29	
Female	1,200	663.40	1736.61	
Onset age				0.03*
<50	660.00	384.48	935.52	
≥50	NA[a]			
Onset symptom				0.52
Extraocular muscle	1,080	535.57	1624.44	
Bulbar muscle or neck or facial muscle	810	0	1674.40	
Limb or trunk	NA[a]			
History of relapse				0.23
Yes	NA[a]			
No	1,080	716.20	1443.80	
Highest MGFA				0.55
I	1,080[b]	NA		
II	1,200	431.46	1968.54	
III	750	NA[a]		
IV	660	203.18	261.78	
V	810	345.36	133.10	
IS therapy				0.02*
IS+	NA[a]			
IS-	750	586.79	913.21	

**With a significant difference; [a]Uncountable because less than 50% of incidents occurred; [b]only 1 patient in this group and taking the maximum survival time.*

Log-rank test revealed a weak association between age and relapse, which disappeared after adjustment in the multivariable Cox regression model ($p = 0.06$). The patients were grouped according to antibody titer and a multiple Cox regression model showed an HR = 1.02, (95% CI, 0.43–2.43, $p = 0.96$) for relapse in the higher antibody titer group after adjustment. There was no statistical difference between the higher and the lower antibody titer group in age at onset, gender, duration of disease, steroid therapy and IS therapy before enrollment (**Supplementary Table_e1**).

Subgroup Analysis

Subgroup analyses are shown in **Figure 3**, in most of the subgroups, IS use was shown to correlate with a reduction in relapse events. Because of the small sample size in each subgroup, interaction analyses were not performed. IS showed better protection in subgroups with longer disease duration before time 0 (>12 months) and MGFA classifications II-V; however, it needs further study whether these results are statistically

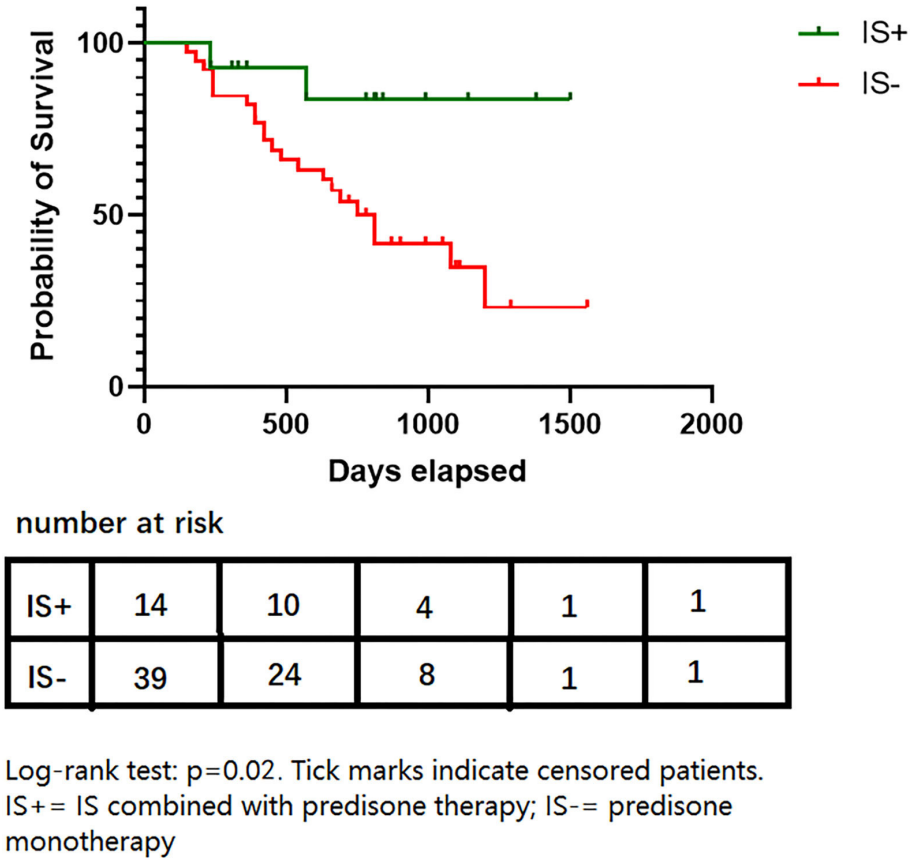


FIGURE 2 | Survival proportions of two groups. Log-rank test: $p = 0.02$. Tick marks indicate censored patients. IS+, IS combined with prednisone therapy; IS-, prednisone monotherapy.

TABLE 3 | Cox regression[a]: analysis for IS therapy and MG relapses.

		Unadjusted		Adjusted[b]	
Group	**Relapse, *n*(%)**	**HR(95%CI)**	***P*-value**	**HR(95%CI)**	***P*-value**
IS+	2(14.3%)	0.21 (0.05–0.58)	0.03	0.23 (0.05–0.93)	0.04
IS-	22(56.4%)	1 (Reference)	NA	1 (Reference)	NA

[a]*Forward: LR;* [b]*Correcting factors include age, gender, relapse history, highest MGFA, and accumulated time of steroid use.*

significant. Data analysis of other subgroups, such as gender, age at onset, different forms of onset, different relapse histories, and different antibody groups showed no association between IS and relapse.

DISCUSSION

In this prospective cohort study of MuSK-positive patients with MG, we observed that long-term IS treatment yielded a 78% reduction in relapse events with a median follow-up of 816 (540, 1110) days. The most prominent AEs were granulocytopenia (AZA-related, 1 case), hair loss (AZA-related, 2 female cases), muscle cramps (TAC-related, 1 case, which disappeared after dose adjustment), and transient elevation of aminotransferases (4 cases, which disappeared after dose adjustment) during the follow-up. In some early studies of oral medication for MG, TAC, MMF, and AZA were discussed as potentially beneficial for MG, with the greatest benefit being the asteroid-sparing effect (18–20). However, the greatest drawback of the above studies is that the types of antibodies studied were not elucidated. MG is a highly heterogeneous group of autoimmune diseases and the subclass to which the antibodies belong will directly affect the outcome of the study; differences between MuSK-MG and AchR-MG in response to immunomodulatory regimens were discussed by several authors (3–5, 21, 22). Our prospective study was designed to offer a new approach to reducing relapse in Chinese patients with MuSK-MG.

Our study showed that 22/39 (56.4%) patients in the subgroup with IS- experienced a relapse, with a significant decrease in relapse events in the IS+ group (14.3%). The median time to relapse in the IS- the group was 22.5 months, and the median time to relapse could not be calculated for the IS+ group as only 2 relapses occurred. Some previous literature did not discuss steroid monotherapy separately from steroids in combination with IS therapy for MuSK-MG, where the proportion of relapse

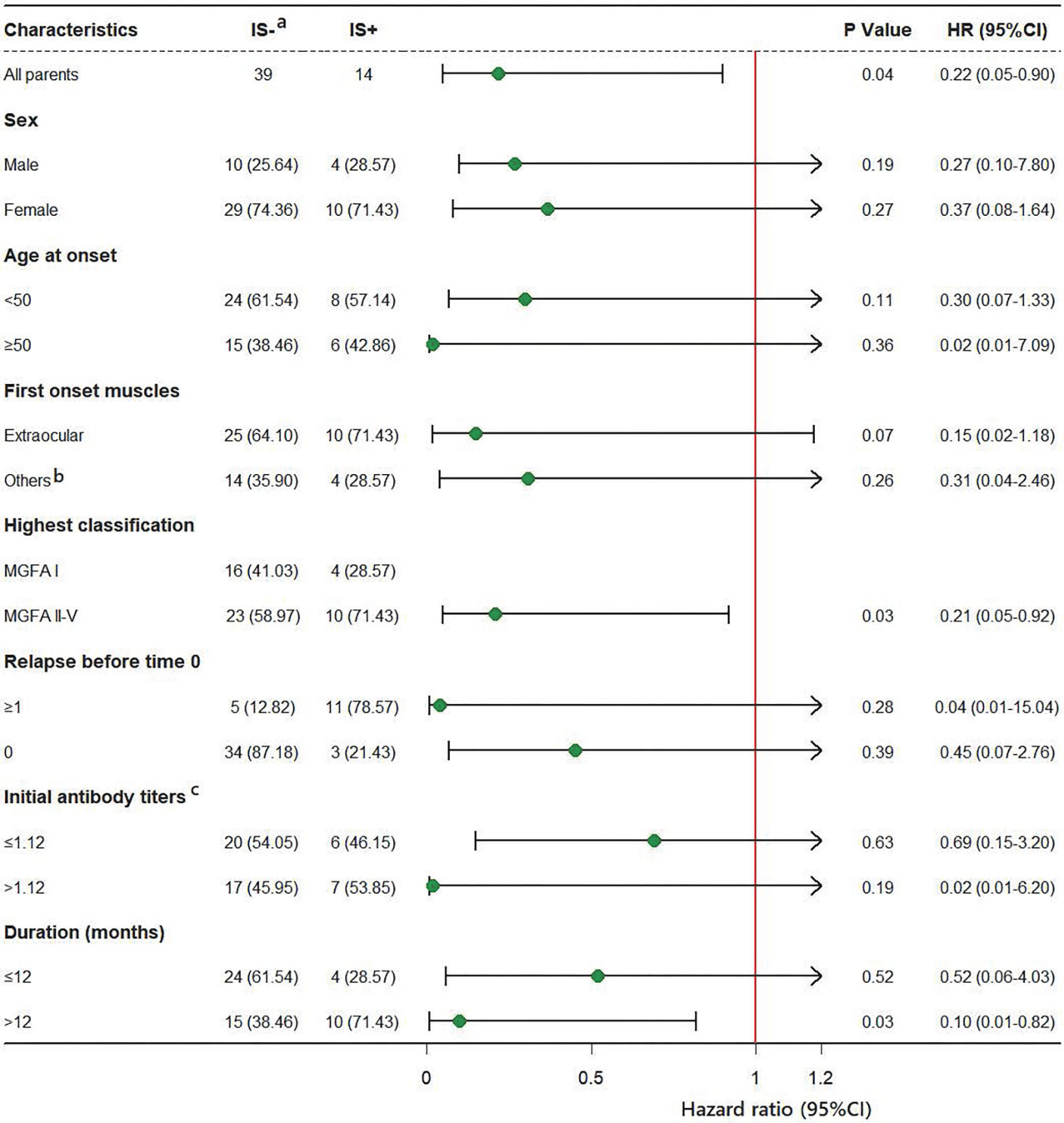


FIGURE 3 | HRs for relapse in subgroup analysis. (a) Data of group IS- used as a reference. (b) Other muscle groups: bulbar muscles, facial muscles, limb muscles and neck muscles are involved. (c) Three missing data points.

was 23 to 40% without subgrouping, and the median time to relapse was 22 months (23, 24). The relapse rate in the IS+ group in this study was lower than that in published studies, we speculated that it was due to the following mechanisms: 1) it was related to the low-dose steroid maintenance therapy in this study and 84.9% of the study subjects were unsuccessful in discontinuing steroid at the end of follow-up; 2) the lower MGFA of the enrolled patients compared to other studies, with 13.2% MGFA-V in this study and a maximum of 47% in other similar studies (23, 25).

It is increasingly recognized that MuSK-MG responded well to steroid treatment, and traditional oral non-steroid IS is not a substitute for long-term steroid maintenance therapy (26, 27); our study showed similar results. Although long-term low-dose

steroid maintenance therapy cannot reduce relapse, it cannot be excluded that it plays a role in the persistence of non-steroid IS. In our study, there was a higher proportion of steroid therapy in the IS+ group at time 0, and inconsistency between individuals in the way steroids and IS were maintained and reduced, which may lead to bias in our findings.

Previously, it was thought that MuSK antibody titers were significantly correlated with disease severity and that MuSK antibody titers could decrease as patients achieved remission (27). We did not find a correlation between higher antibody titers and the probability of relapse, and there were no significant differences for various factors such as duration of steroid use, the median time to relapse, steroid daily dose at the end of follow-up, or MGC score and MG-ADL at the end of follow-up between different antibody titer subgroups. We speculate that antibodies may be a concomitant manifestation of the active state of the disease.

Our cohort study showed that more than half of MuSK-Patients with MG achieved minimal manifestation (MM) and better at the end of follow-up (**Table 1B**), which is consistent with the findings in a previous study [PIS-MM and better = 5/21(23.8%]) PIS-I = 13/21(61.9%)] (23). It is worth noting that our study specified that IVIG or methylprednisolone pulse therapy was only applied when the Man G crisis occurred, and no patients had received PLEX or rituximab, which may have led to a more conservative perception of the study results.

Our study has several limitations. Firstly, this is an observational real-world study, randomization was not applied to group patients, and the results could be biased by baseline characteristics. Secondly, the number of patients enrolled is smaller compared to the ideal model, which may introduce additional bias to the study conclusion and subgroup analysis. We should be more cautious in interpreting the results of data analysis, and relevant findings need to be confirmed in future studies involving a larger patient population. Thirdly, we cannot exclude role for the steroid in the effect of IS. Finally, considering the effects of non-steroid IS on women of reproductive age, the pros and cons need to be weighed in practical application.

CONCLUSIONS

Our study adds to the current information available on MuSK-MG treatment, which has been hampered by the small number of studies and many methodological flaws, suggesting that non-steroid IS use is the only factor associated with relapse. In addition, we found that MuSK-MG with a longer duration and a general manifestation (MGFA II and even severer MGFA) may have a better response to treatment with non-steroid IS, which was not reported in previous studies. Furthermore, we reviewed this large cohort at our institution to evaluate the clinical course and long-term outcomes of Chinese MuSK-MG populations. It is hoped that a prospective randomized trial will be available in the future to observe the efficacy and safety of IS in the treatment of MuSK-MG and that the exploration of the specific mechanisms of IS onset will be an important task to be addressed in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, China. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YT contributed to drafting and revising the manuscript, study concept and design, acquisition of data, and statistical analysis. LZ contributed to the lab work. YH, KL, JY, and JS contributed to the acquisition of data and interpretation of the data. YG and LC contributed to drafting and revising the manuscript, study concept and design, and interpretation of the data. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Beijing Municipal Sciences and Technology Commission (Z181100001718145), National Clinical Cohort Study of Rare Diseases Program (2016YFC0901501), and the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2019XK320039).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.877895/full#supplementary-material

REFERENCES

1. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med*. (2001)7:365–8 doi: 10.1038/85520
2. DeChiara TM, Bowen DC, Valenzuela DM, Simmons MV, Poueymirou WT, Thomas S, et al. The receptor tyrosine kinase MuSK is required for neuromuscular junction formation in vivo. *Cell*. (1996) 85:501–12. doi: 10.1016/S0092-8674(00)81251-9
3. Evoli A, Alboini PE, Bisonni A, Mastrorosa A, Bartocccioni E. Management challenges in muscle-specific tyrosine kinase myasthenia gravis. Annals of the New York Academy of Sciences. (2012)1274: 86–91. doi: 10.1111/j.1749-6632.2012.06781.x
4. Cao M, Koneczny I, Vincent A. Myasthenia gravis with antibodies against muscle specific kinase: an update on clinical features, pathophysiology and

treatment. *Front Mol Neurosci.* (2020) 13:159. doi: 10.3389/fnmol.2020.00159
5. Rodolico C, Bonanno C, Toscano A, Vita G. MuSK-associated myasthenia gravis: clinical features and management. *Front Neurol.* (2020) 11:660. doi: 10.3389/fneur.2020.00660
6. Evoli A, Bianchi MR, Riso R, Minicuci GM, Batocchi AP, Servidei S, et al. Response to therapy in myasthenia gravis with anti-MuSK antibodies. *Ann N Y Acad Sci.* (2008) 1132:76–83. doi: 10.1196/annals.1405.012
7. Hehir MK, Burns TM, Alpers J, Conaway MR, Sawa M, Sanders DB. Mycophenolate mofetil in AChR-antibody-positive myasthenia gravis: outcomes in 102 patients. *Muscle Nerve.* (2010) 41:593–8. doi: 10.1002/mus.21640
8. Cruz JL, Wolff ML, Vanderman AJ, Brown JN. The emerging role of tacrolimus in myasthenia gravis. *Ther Adv Neurol Disord.* (2015) 8:92–103. doi: 10.1177/1756285615571873
9. Sathasivam S. Current and emerging treatments for the management of myasthenia gravis. *Ther Clin Risk Manag.* (2011) 7:313–23. doi: 10.2147/TCRM.S14015
10. Fan Z, Li Z, Shen F, Zhang X, Lei L, Su S, et al. Favorable effects of tacrolimus monotherapy on myasthenia gravis patients. *Front Neurol.* (2020) 11:594152. doi: 10.3389/fneur.2020.594152
11. Zhou L, Liu W, Li W, Li H, Zhang X, Shang H, et al. Tacrolimus in the treatment of myasthenia gravis in patients with an inadequate response to glucocorticoid therapy: randomized, double-blind, placebo-controlled study conducted in China. *Ther Adv Neurol Disord.* (2017)10: 315–25. doi: 10.1177/1756285617721092
12. Pasnoor M, He J, Herbelin L, Burns TM, Nations S, Bril V, et al. A randomized controlled trial of methotrexate for patients with generalized myasthenia gravis. *Neurology.* (2016) 87:57–64. doi: 10.1212/WNL.0000000000002795
13. Heckmann JM, Rawoot A, Bateman K, Renison R, Badri M, A. single-blinded trial of methotrexate versus azathioprine as steroid-sparing agents in generalized myasthenia gravis. *BMC Neurol.* (2011) 11:97. doi: 10.1186/1471-2377-11-97
14. Vincenzo D, Antonino L, Marianna Gabriella R, Antonio D, Filippo B, Carmelo R. Rituximab in AChR subtype of myasthenia gravis: systematic review. *J Neurol Neurosurgery Psychiatry.* (2020) 10:1–4.
15. Guptill JT, Sanders DB, Evoli A. Anti-MuSK antibody myasthenia gravis: clinical findings and response to treatment in two large cohorts. *Muscle Nerve.* (2011) 44:36–40. doi: 10.1002/mus.22006
16. Lavrnic D, Losen M, Vujic A, De Baets M, Hajdukovic L J, Stojanovic V, et al. The features of myasthenia gravis with autoantibodies to MuSK. *J Neurol Neurosurg Psychiatry.* (2005) 76:1099–102. doi: 10.1136/jnnp.2004.052415
17. Burns TM. The MG composite: an outcome measure for myasthenia gravis for use in clinical trials and everyday practice. *Ann N Y Acad Sci.* (2012) 1274:99–106. doi: 10.1111/j.1749-6632.2012.06812.x
18. Minami N, Fujiki N, Doi S, Shima K, Niino M, Kikuchi S, et al. Five-year follow-up with low-dose tacrolimus in patients with myasthenia gravis. *J Neurol Sci.* (2011) 300:59–62. doi: 10.1016/j.jns.2010.09.033
19. Ben Younes T, Benrhouma H, Klaa H, Ben Aoun R, Rouissi A, Ben Ahmed M, et al. Muscle-specific kinase autoimmune myasthenia gravis: report of a pediatric case and literature review. *Neuropediatrics.* (2011) 300:59–62.
20. Muscle Study Group. A trial of mycophenolate mofetil with prednisone as initial immunotherapy in myasthenia gravis. *Neurology*. (2008) 71:394–9. doi: 10.1212/01.wnl.0000312373.67493.7f
21. Zhao S, Zhang K, Ren K, Lu J, Ma C, Zhao C, et al. Clinical features, treatment and prognosis of MuSK antibody-associated myasthenia gravis in Northwest China: a single-centre retrospective cohort study. *BMC Neurol.* (2021) 21:315–25. doi: 10.1186/s12883-021-02439-7
22. Borges L S, Richman D P. Muscle-Specific Kinase Myasthenia Gravis. *Front Immunol.* (2008) 71:400–6. doi: 10.3389/fimmu.2020.00707
23. Evoli A, Tonali PA, Padua L, Monaco ML, Scuderi F, Batocchi AP, Marino M, Bartoccioni E. Clinical correlates with anti-MuSK antibodies in generalized seronegative myasthenia gravis. *Brain*. (2003) 126(Pt 10):2304–11. doi: 10.1093/brain/awg223
24. Brauner S, Eriksson-Dufva A, Hietala M A, Frisell T, Press R, Piehl F. Comparison between rituximab treatment for new-onset generalized myasthenia gravis and refractory generalized myasthenia Gravis. *JAMA Neurol.* (2020) 77:974–81. doi: 10.1001/jamaneurol.2020.0851
25. Diaz-Manera J, Martinez-Hernandez E, Querol L, Klooster R, Rojas-Garcia R, Suarez-Calvet X, et al. Long-lasting treatment effect of rituximab in MuSK myasthenia. *Neurology.* (2012) 78:189–93. doi: 10.1212/WNL.0b013e3182407982
26. Mantegazza R, Cavalcante P. Diagnosis and treatment of myasthenia gravis. *Curr Opin Rheumatol.* (2019) 31:623–33. doi: 10.1097/BOR.0000000000000647
27. Bartoccioni E, Scuderi F, Minicuci G M, Marino M, Ciaraffa F, Evoli A. Anti-MuSK antibodies: correlation with myasthenia gravis severity. *Neurology.* (2006) 67:505–7. doi: 10.1212/01.wnl.0000228225.23349.5d

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 21 June 2022
doi: 10.3389/fneur.2022.833062

LncRNA LINC00680 Acts as a Competing Endogenous RNA and Is Associated With the Severity of Myasthennia Gravis

Li Liu[1,2], Huixue Zhang[1], Xiaoyu Lu[1], Lifang Li[1], Tianfeng Wang[1], Shuang Li[1], Xu Wang[1], Si Xu[1], Lei Li[1], Qian Li[1], Tingting Yi[1], Tao Wu[3], Zhimin Chen[1], Hongyu Gao[1], Jianjian Wang[1] and Lihua Wang[1]**

[1] Department of Neurology, The Second Affiliated Hospital of Harbin Medical University, Harbin, China, [2] Department of Neurology, Heilongjiang Provincial Hospital, Harbin, China, [3] Department of Neurology, Xuanwu Hospital of Capital Medical University, Beijing, China

OPEN ACCESS

Edited by:
Nils Erik Gilhus,
University of Bergen, Norway

Reviewed by:
Jing Li,
Central South University, China
Jie Zhu,
Karolinska University Hospital Solna, Sweden
Liis Sabre,
University of Tartu, Estonia

***Correspondence:**
Jianjian Wang
wangjian_427@163.com
Lihua Wang
wanglh211@163.com

Specialty section:
This article was submitted to Neuromuscular Disorders and Peripheral Neuropathies, a section of the journal Frontiers in Neurology

Received: 10 December 2021
Accepted: 16 May 2022
Published: 21 June 2022

Citation:
Liu L, Zhang H, Lu X, Li L, Wang T, Li S, Wang X, Xu S, Li L, Li Q, Yi T, Wu T, Chen Z, Gao H, Wang J and Wang L (2022) LncRNA LINC00680 Acts as a Competing Endogenous RNA and Is Associated With the Severity of Myasthennia Gravis. Front. Neurol. 13:833062. doi: 10.3389/fneur.2022.833062

Background and Purpose: Myasthenia gravis (MG) is a T cell-dependent antibody-mediated autoimmune disorder that can seriously affect patients' quality of life. However, few studies have focused on the severity of MG. Moreover, existing therapeutic efforts, including those targeting biomarkers for MG, remain unsatisfactory. Therefore, it is vital that we investigate the pathogenesis of MG and identify new biomarkers that can not only evaluate the severity of the disease but also serve as potential therapeutic targets. Long noncoding RNA LINC00680 has been found to be associated with the progression of a variety of diseases as a competing endogenous RNA (ceRNA). However, the specific role of LINC00680 in MG has yet to be clarified. Here, we aimed to investigate the association between LINC00680 and the severity of MG.

Methods: Bioinformatics tools, quantitative real-time PCR, Western blotting, and luciferase assays were selected to investigate key signaling pathways and RNA expression in patients with MG. The Quantitative MG Score scale and the MG Composite scale were used to evaluate the severity of MG in the included patients. Cell viability assays and flow cytometry analysis were selected to analyze cell proliferation and apoptosis.

Results: Compared with control subjects, the expression levels of LINC00680 and mitogen-activated protein kinase 1 (MAPK1) in peripheral blood mononuclear cells of patients with MG were both upregulated; the levels of miR-320a were downregulated. A positive correlation was detected between LINC00680 expression and the severity of MG. Luciferase reporter assays identified that LINC00680 acts as a target for miR-320a. The *in vitro* analysis confirmed that LINC00680 regulates the expression of MAPK1 by sponging miR-320a. Finally, the functional analysis indicated that LINC00680 promoted Jurkat cell proliferation and inhibited cellular apoptosis by sponging miR-320a.

Conclusion: LINC00680 may be associated with the severity of MG as a ceRNA by sponging miR-320a to upregulate MAPK1. These findings suggest that LINC00680 may

represent a potential biomarker which evaluates the severity of MG and may serve as a therapeutic target.

Keywords: LINC00680, severity, myasthenia gravis, competing endogenous RNA (ceRNA), biomarker

INTRODUCTION

Myasthenia gravis (MG) is a T cell-dependent antibody- and complement-mediated autoimmune disease that affects the function of neuromuscular junctions (1). The manifestation of this disease is a fluctuating weakness of the skeletal muscles (2–4). This weakness occurs proximally more often than distally and can be localized or systemic. Furthermore, the eye muscles are almost always affected by muscle weakness and are accompanied by diplopia and ptosis (3). The most typical characteristic of MG is that the weakness becomes more apparent with exercise and a repeated use of the muscles (fatigue) and varies at different times of day; in fact, strength is normal in the morning and weaker in the afternoon or evening (5). Various types of antibodies are involved, predominantly acetylcholine receptor (AChR) antibodies, muscle-specific kinase antibodies, and lipoprotein receptor-related protein 4 antibodies; of these, acetylcholine receptor antibodies are the most important (6). Anti-AChR antibody has a high-affinity and pathogenic immunoglobulin G (IgG); the synthesis of this antibody requires interaction between activated CD4+ T cells and B cells. CD4+ T cells, and their related cytokines, are critical for the progression of MG symptoms (7). Cytokines are crucial to the production of autoantibodies and cellular immune regulation of MG. Proinflammatory cytokines secreted by T-helper 1 (Th1) cells, such as interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α), are responsible for the differentiation and growth of B cells that synthesize immunoglobulin isotypes (7–10). In addition, IFN-γ can also stimulate major histocompatibility complex (MHC) class II molecule expression on the membrane of muscle cells, which facilitates the presentation of muscle cells AChR (7). Some of the cytokines secreted by Th2 cells, such as IL-4 and IL-10, are vital factors that can also affect the differentiation and growth of B cells and stimulate an immune response (11). According to statistical surveys, the annual incidence of MG is 8–10 per 1 million people and the prevalence is 150–250 per 1 million people (12). MG can have serious effects on the quality of patients' life. Nevertheless, so far, there is a notable lack of biomarkers to evaluate the clinical severity of MG; moreover, the efficacy of clinical treatments is not satisfactory (13, 14). MG is still associated with a high recurrence rate; the condition of many patients continues to worsen over time (15). Therefore, it is vital for us to investigate the specific mechanisms involved and identify new biomarkers that not only can evaluate the severity of MG but can also be used as therapeutic targets. Recent studies have reported the fact that many non-coding RNAs (ncRNAs) have been involved in the regulation of genes during the disease process in the immune system and their role was quite vital (16). This offers a new direction for identifying new markers for MG.

Long non-coding RNAs (lncRNAs), as a widely studied form of ncRNA, are a type of RNA molecule with a length of no <200 nucleotides and have no or little protein-coding function (17). Studies have shown that lncRNAs exert regulatory functions in a diverse array of biological processes, and in mechanisms of disease; these effects are known to be associated with the subcellular localization of lncRNAs. In general, cytoplasmic lncRNAs mainly regulate posttranscriptional events, while nuclear lncRNA modulates transcriptional processes (18, 19). Studies suggest that lncRNA functions *via* three mechanisms: interaction with other RNAs, interaction with chromatin, and interaction with proteins (20). Furthermore, some studies have also shown the fact that lncRNAs have been involved in the occurrence and development of immune system disorders such as multiple sclerosis (21), ankylosing spondylitis (22), and systemic lupus erythematosus (23). Besides, it has been reported that lncRNA IFNG-AS affects the activity of CD4+ T cells by influencing HLA-DRB1 expression in MG (24). Another study has shown that lncRNA XLOC_003810 promotes T-cell activation and inhibits programmed death-1/programmed death ligand-1 (PD-1/PD-L1) expression in patients with MG-related thymoma (25).

Over the past few years, more and more studies have investigated the dysregulation of the lncRNA-microRNA (miRNA)-messenger RNA (mRNA) network according to the competing endogenous RNA (ceRNA) theory. The ceRNA studies have revealed a new mechanism for RNA–RNA interactions, communication, and coregulation, in which miRNAs can affect gene expression by binding to mRNAs. The study has shown that miRNAs bind to partial complementarity sequences within transcripts of target RNA by miRNA recognition elements (MREs). Furthermore, lncRNAs could regulate the activity of miRNAs on their target mRNAs by acting as sponges for miRNAs *via* the MREs (26). As a ceRNA, lncRNA competes with mRNA target to bind to miRNA so as to reduce the inhibiting effect of miRNA on mRNA target in a great diversity of diseases (27, 28). For example, the lncRNA JPX transcript, XIST activator (JPX) was shown to upregulate the Twist1 expression by sponging miRNA-33a-5p to regulate the growth and metastasis of lung cancer (29). The lncRNA BCRT1 acts as ceRNA and regulates polypyrimidine tract binding protein 3 (PTBP3) expression by targeting miR-1303 in breast carcinoma (30). However, the specific effects of lncRNAs as ceRNAs in MG are still largely unknown. LINC00680 is a newly discovered lncRNA that is mainly localized to the cytoplasm of cells, as determined by RNALocate (http://www.rna-society.org/rnalocate/) (31). Indeed, an increasing body of evidence now indicates that LINC00680 serves as a ceRNA and is related to the development of a variety of carcinomas. For example, LINC00680 activates AKT serine/threonine kinase 3 (AKT3) by sponging miR-568, thus promoting stemness properties and decreasing chemosensitivity in hepatocellular carcinoma stemness (HCCs) (32). LINC00680 functions as a sponge of miR-410-3p to enhance high mobility group box 1 (HMGB1)

TABLE 1 | Characteristics of patients with myasthenia gravis (MG) and healthy controls.

Characteristic	MG ($n = 31$)	control ($n = 31$)
Age (y)	55.71±16.13	56.29 ±12.88
Gender (M/F)	13/18	15/16
Age of onset (y)		
EOMG(≤ 50 y)	12	–
LOMG(>50 y)	19	–
AChR Ab (Positive/Total)	20/24	–
Thymoma		
Yes	13	–
No	18	–
Subgroups		
OMG	14	–
GMG	17	–
History of the disease	No	No
Infectious disease	No	No
Other associated active autoimmune diseases		
Treatment in 1 month prior to consultation		
Corticosteroids	NO	NO
Immunosuppressants	No	NO
Intravenous immunoglobulins	No	NO
Plasma exchange	No	NO

Among the 31 patients with MG, 24 patients had MG-related antibody test and 7 patients refused MG-related antibody test.

TABLE 2 | The primers used for real-time PCR.

Name	Sequences
LINC00680 Forward primer (5′->3′)	GTGGAACCTCAGGCATCCA
LINC00680 Reverse primer (5′->3′)	TATACACAGAGAGGGAGAAAGAC
miR-320a Forward primer (5′->3′)	AAAAGCUGGGUUGAGAGGGCGA
GAPDH Forward primer (5′->3′)	GAGAAGTATGACAACAGCCTCAA
GAPDH Reverse primer (5′->3′)	GCCATCACGCCACAGTTT

expression to promote the progression of non-small cell lung cancer (33). The specific role of LINC00680 in MG is still not clarified.

In this study, we systematically identified the changes of LINC00680 expression in patients with MG and compared the expression with those from control subjects; this allowed us to correlate expression changes with the severity of MG. Then, we predicted the LINC00680-miR-320a-MAPK1 ceRNA network through bioinformatics tools and by reviewing reliable studies. Finally, biological experiments were designed to confirm the existence of the LINC00680-miR-320a-MAPK1 ceRNA network. Our results highlight the novel role of LINC00680 as a ceRNA and show that this form of LINC00680 is associated with the severity of MG by sponging miR-320a and upregulating MAPK1, thus providing new insights into the specific roles of LINC00680 in MG.

MATERIALS AND METHODS

Patients and Clinical Data

In this study, blood samples were taken from a cohort of patients with MG ($n = 31$; 18 women and 13 men) and normal control volunteers ($n = 31$; 16 women and 15 men) who attended the Second Affiliated Hospital of Harbin Medical University. All the patients met the criteria for diagnosing MG. We excluded patients with a history of infectious disease and other associated active autoimmune diseases, patients receiving treatment with corticosteroids, immunosuppressants, intravenous immunoglobulins, or plasma exchange 1 month before consultation, pregnant women, and patients with psychiatric comorbidities or cognitive conditions that prevented the measurement of the key parameters being evaluated. The mean age of the MG group was 55.71 ± 16.13 years, while the mean age of the control group was 56.29 ± 12.88 years. An age of 50 years was used as a threshold to distinguish early-onset MG (EOMG) (≤50 years) from late-onset MG (LOMG) (>50 years). A part of the clinical data of all the patients and healthy controls are shown in **Table 1**. The Ethics Committee of the Second Affiliated Hospital of Harbin Medical University approved this study. All the subjects provided written informed consent. This study followed the provisions of the Declaration of the World Medical Association in Helsinki.

Data relating to the age at onset, age, gender, and AChR Ab were collected from all the subjects. Detailed neurological examinations were performed using the Quantitative MG Score (QMGs) scale and the MG Composite (MGC) scale. The detailed information on the severity of patients with MG is shown in **Supplementary Table 1**.

Clinical Samples

Peripheral blood was collected from all the subjects in a test tube containing ethylenediaminetetraacetic acid (EDTA), and a lymphocyte separation solution was used to distill mononuclear cells from the peripheral blood mononuclear cells (PBMCs). Then, the cells were kept in a refrigerator at −80°C for subsequent use.

Bioinformatics Analysis

StarBase v2.0 (http://starbase.sysu.edu.cn/) (34) and DIANA-LncBase (http://carolina.imis. athena-innovation.gr/diana_tools/web/index.ph) (35) were used to predict the miRNAs that might bind to the identified lncRNA. The Nervous System Disease NcRNAome Atlas (NSDNA) prioritization tool (http://bio-bigdata.hrbmu.edu.cn/nsdna/search.jsp) (36) was also used to identify specific miRNAs that may be associated with MG.

RNA Extraction, Reverse Transcription, and qRT-PCR

Trizol Reagent (Sigma Life Science, Darmstadt, Germany) was selected to distill total RNAs from PBMCs referring to the manual. The Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland) was selected to reverse-transcribed

total RNA with corresponding primers referring to the manual; this allowed us to detect the expression levels of LINC00680 and MAPK1. The miRcute Plus miRNA First-Strand cDNA Kit (Tiangen Biotech, Beijing, China) was selected to reverse-transcribed total RNA using corresponding primers and referring to the manual for the subsequent detection of miR-320a expression levels. The FastStar Universal SYBR Green Master Kit (Roche, Basel, Switzerland) was then used to detect the expression levels of LINC00680 and MAPK1 by quantitative real-time PCR (qRT-PCR). The miR-320a expression level was measured by qRT-PCR using the miRcute Plus miRNA qPCR Kit and SYBR Green (Tiangen Biotech, Beijing, China). We choose glyceraldehyde-3-phosphate dehydrogenase (GAPDH), U6 was used as an internal control, and target gene expression levels were normalized by the $2^{-\Delta\Delta CT}$ method. The sequences of primers are given in **Table 2**.

Cell Culture

Jurkat cells line and 293T cells line were both purchased from the American Type Culture Collection (Manassas, VA, USA), grown in a basic Roswell Park Memorial Institute (RPMI) 1640 medium (Thermo Fisher Scientific, Beijing, China) containing 10% serum of fetal bovine serum (Excell Bio, Suzhou, China) and 1% penicillin/streptomycin (Beyotime Biotechnology, Nanjing, China), and cultured in a suitable incubator (37°C, 5% CO_2, and saturated humidity). The fresh medium was used to replace the old medium every 1 or 2 days according to the status of cell growth; cells that were in the logarithmic growth stage were used to conduct the subsequent experiments.

Cell Transfection

A negative control (NC), miR-320a mimics, and miR-320a inhibitor were obtained from General Biol (Anhui, China) and transfected into Jurkat cells using Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA) referring to the manual. Then, a lncRNA Smart Silencer for human LINC00680 was purchased from Ribobio (Guangdong, China). Lipofectamine® 2000 was selected to transfect Jurkat cells with specific plasmids, as described previously. The sense and antisense sequences for the LINC00680 Smart Silencers were as follows: siLINC00680-1, 5′-TCCATTCATTGGGAAATCA-3′ and 5′-AGGGCAGTGTG GAGTGACA-3′; siLINC00680-2, 5′-CATGGACAATATCA TAGTT-3′ and 5′-CCTCAGCTCTCCATGGCTCT-3′; and siLINC00680-3, 5′-AGTGTGGAGTGACAGGCACG-3′ and 5′-AAGCATCCATTCATTGGGAA-3′. Cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C. Total RNA was then extracted from the transfected cells and analyzed by qRT-PCR to assess the transfection efficiency.

Dual-Luciferase Reporter Assays and Cell Culture

Established protocols were used to perform a dual-luciferase reporter assay to investigate the binding activity between LINC00680 and miR-320a in 293T cells. The binding site for LINC00680 and miR-320a was predicted by the bioinformatics website (http://starbase.sysu.edu.cn/). To construct a LINC00680-WT luciferase reporter vector, we cloned a fragment of LINC00680 that contains the predicted binding site of miR-320a into the PHY-811 vector (Hanyi Biotechnology, Shanghai, China). Next, we mutated the assumed binding site for miR-320a in LINC00680, creating a vector that we named LINC00680-MUT. The LINC00680-WT or LINC00680-MUT vector was then transfected into 293T cells in combination with negative control or miR-320a mimics using Lipofectamine® 2000. After 48 h of transfection, luciferase activity was determined using a dual-luciferase reporting analysis system (Promega, WI, USA) referring to the manual. Relative luciferase activity was measured and normalized to renin luciferase activity. These experiments were replicated three times in total.

Western Blot Analysis

Protease inhibitors (Beyotime Biotechnology, Nanjing, China) and radio immunoprecipitation assay (RIPA) buffer (Beyotime Biotechnology, Nanjing, China) were used to distill total protein from Jurkat cells. Total protein concentration was measured using the Bicinchoninic Acid Protein Assay Kit (Beyotime Biotechnology, Nanjing, China). Protein extracts were then dissolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore, MA, USA) by a semidry transfer way. Next, the membranes were blocked by a sealed liquid at room temperature for 0.5 h and then washed with Tris-buffered saline-Tween-20 (TBST). Then, the membranes were incubated with MAPK1 (1:1,000) and GAPDH (1:1,000) primary antibodies at 4°C overnight. The following morning, the membranes were washed with TBST and incubated with horseradish peroxidase-labeled secondary antibody immunoglobulin G (IgG) (1:1,000) for 2 h at room temperature. The PVDF membranes were then rewashed in TBST, and the Enhanced Chemiluminescence (ECL) Kit (Beyotime Biotechnology, Nanjing, China) was used to visualize positive immunobinding. GAPDH was selected as the internal control. These experiments were replicated three times in total.

CCK-8 Assays

Cell Counting Kit-8 (CCK8) assays (Dojindo, Tokyo, Japan) were selected to measure cell proliferation. Cells that have been transfected with siLINC00680, negative control, and siLINC00680, along with miR-320a inhibitor were grown at a density of 1,500 cells/well in 96-well plates and incubated in an atmosphere containing 5% CO_2 at 37°C and with saturated humidity. Then, 10 μl of CCK-8 reagent was added to each well at 24, 48, 72, and 96 h after transfection and incubated at 37°C for 2 h. The absorbance at 450 nm was then detected from each well. These experiments were repeated independently in triplicate.

Flow Cytometry Analysis

Cell apoptosis was determined with the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) Apoptosis Detection Kit (BD Biosciences, San Jose, CA, USA) in accordance with the instructions. Jurkat cells were transfected with negative control, siLINC00680, and siLINC00680, along with a miR-320a inhibitor, and were cultured in a six-well plate at 5% CO_2 at

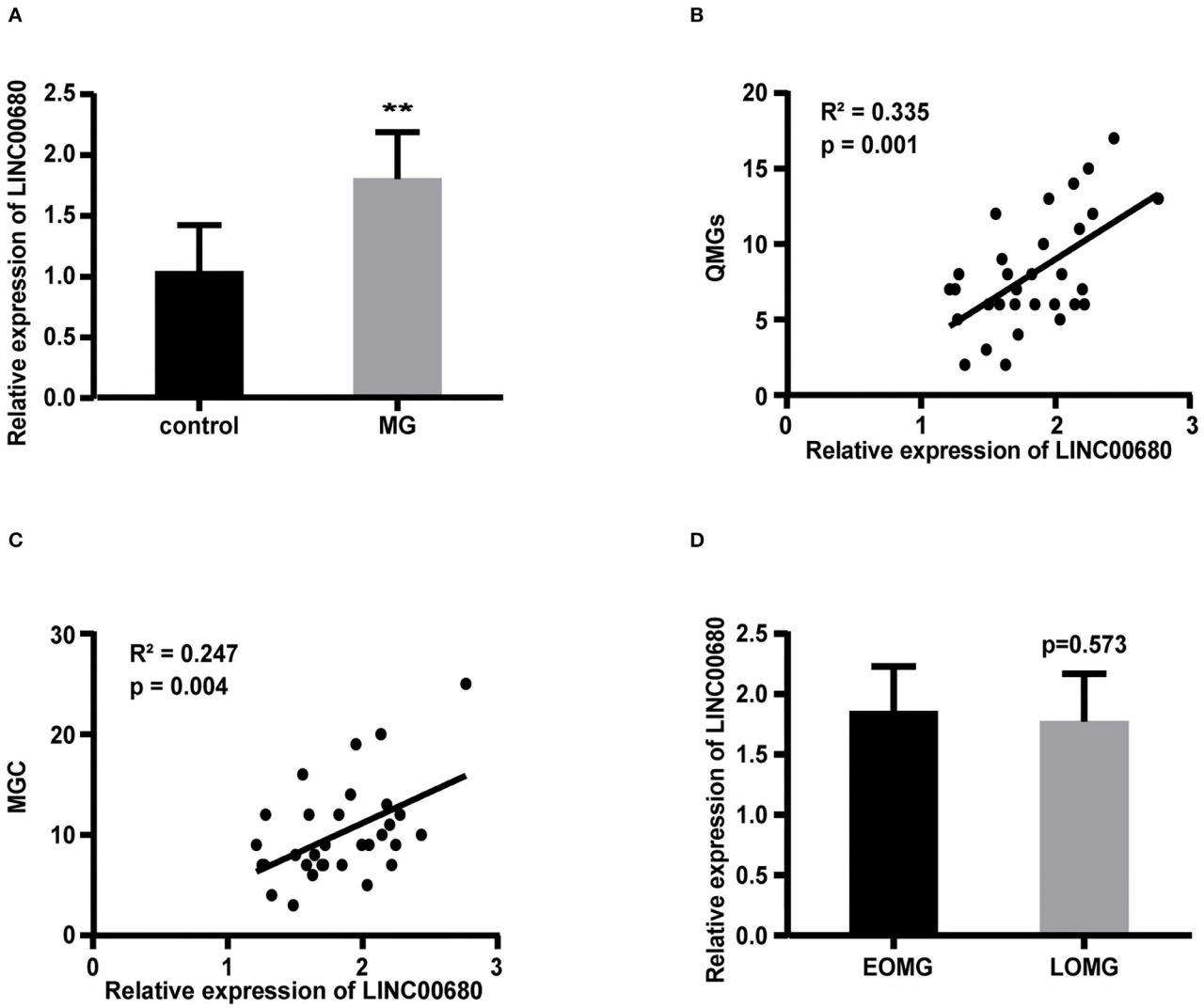


FIGURE 1 | Expression of LINC00680 in MG and its correlation with MG severity and onset age. **(A)** LINC00680 expression was investigated in 31 patients with MG and 31 control subjects by real-time PCR **(B)** Correlation analyses between QMGs and LINC00680 expression in patients with MG **(C)** Correlation analyses between MGC and LINC00680 expression in patients with MG **(D)** LINC00680 expression did not differ significantly between early-onset MG (EOMG) and late-onset MG (LOMG) ($^{**}p < 0.01$). MG, myasthenia gravis; QMGs, Quantitative MG score; MGC, Myasthenia Gravis Composite.

37°C and with saturated humidity for 48 h. Subsequently, the cells were collected and washed with phosphate-buffered saline. Then, the cells were stained with annexin V-FITC and PI in the dark and the stained cells were measured by flow cytometry and analyzed by CellQuest software. These experiments were repeated in triplicate.

Statistical Analysis

Experiments were replicated three times in total. SPSS software version 23.0 and GraphPad Prism version 8.0 were selected for statistical analyses. The continuous variables were shown as mean ± SD. Comparisons between the two groups were analyzed by the Student's *t*-test, and the multiple groups were analyzed by ANOVA. We also performed Pearson's correlation analysis. The *p*-value <0.05 indicated statistical significance.

RESULTS

LINC00680 Was Upregulated in MG and Was Associated With the Severity of MG

Quantitative real-time PCR (qRT-PCR) was selected to measure the LINC00680 expression levels in PBMCs of patients with MG and normal controls. Our results demonstrated that LINC000680 expression of patients with MG was at notably higher levels than that of normal controls ($p < 0.01$; **Figure 1A**). In addition, we investigated the association between the expression of LINC00680 and the severity of MG. There was a correlation between higher expression levels of LINC00680 with higher scores on the QMGs ($R^2 = 0.335$, $p = 0.001$; **Figure 1B**). Moreover, the LINC00680 expression levels were also correlated with the MGC score

($R^2 = 0.247$, $p = 0.004$; **Figure 1C**). However, no obvious difference was found between EOMG and LOMG with regard to the expression levels of LINC00680 ($p = 0.573$; **Figure 1D**).

Construction of a LINC00680-miR-320a-Mitogen-Activated Protein Kinase 1 Interaction Network in MG

We predicted 34 miRNAs associated with LINC00680 through the DIANA-LncBase and 6 miRNAs associated with LINC00680 through the Starbase. We also identified 131 miRNAs that were associated with MG from the NSDNA. We identified two miRNAs at the intersection of these three sets of miRNAs; miR-320a was one of these two miRNAs (**Figure 2A**). A previous study reported that miR-320a was not only downregulated in patients with MG but was also mediated by the regulation of MAPK1 by directly targeting MAPK1 in Jurkat cells (1). In addition, the targeting relationship between miR-320 and MAPK1 has been verified by dual-luciferase reporter assays in a previous study (1). Therefore, we selected miR-320a and MAPK1 to construct an interaction network with LINC00680. In this study, the miR-320a and MAPK1 expression levels were also analyzed by qRT-PCR in patients with MG and normal controls. We discovered that miR-320a was downregulated and MAPK1 was upregulated in MG ($p < 0.01$, **Figure 2B**; $p < 0.01$, **Figure 2C**); these results were consistent with the previous study (1). In addition, we detected the association between the expression levels of miR-320a and MAPK1 and the association between the expression levels of LINC00680 and MAPK1 in patients with MG. We found that there was a negative correlation between miR-320a and MAPK1 and a positive correlation between LINC00680 and MAPK1, further confirming the interaction network we had identified ($R^2 = 0.600$, $p < 0.001$, **Figure 2D** $R^2 = 0.335$, $p = 0.001$, **Figure 2E**).

LINC00680 Represents a Target for miR-320a

Bioinformatics analysis revealed that the LINC00680 sequence contained a hypothetical miR-320a-binding region. To investigate the relationship between miR-320a and LINC00680, we transfected Jurkat cells with a miR-320a mimic. qRT-PCR was selected to detect transfection efficiency ($p < 0.01$; **Figure 3A**). The miR-320a overexpression inhibited the expression of LINC00680 ($p < 0.05$; **Figure 3B**). To identify the precise interaction between LINC00680 and miR-320a, we constructed LINC00680 wild-type (WT) and LINC00680 mutant (MUT) luciferase reporter vectors (**Figure 3C**). LINC00680-wt or LINC00680-mut and miR-320a mimic or negative control were co-transfected into 293T cells. Dual-luciferase reporter assays demonstrated that the luciferase activity of LINC00680-WT was repressed by the miR-320a mimic, although the luciferase activity of the LINC00680-MUT was not affected (**Figure 3D**). These findings indicated that LINC00680 is the target of miR-320a.

LINC00680 Modulated MAPK1 Expression by Sponging miR-320a

To detect whether LINC00680 regulates MAPK1 expression by sponging miR-320a, we first transfected Jurkat cells with miR-320a mimics or negative controls to determine the expression levels of MAPK1 protein and mRNA by Western blotting and qRT-PCR, respectively. qRT-PCR was selected to measure the transfection efficiency of the miR-320a mimic ($p < 0.01$; **Figure 3A**). The analysis indicated that the miR-320a overexpression reduced the MAPK1 expression at the mRNA and protein levels ($p < 0.01$, **Figure 4A**; $p < 0.01$, **Figure 4B**), which was consistent with the previous study (1). Then, we transfected Jurkat cells with negative control, siLINC00680, and siLINC00680, along with the miR-320a inhibitor. The MAPK1 mRNA and protein levels were then determined by qRT-PCR and Western blotting. Analysis indicated that the knockout of LINC00680 inhibited the MAPK1 mRNA and protein expression levels in Jurkat cells, while miR-320a inhibitors blocked the siLINC00680-induced reduction in MAPK1 expression ($p < 0.01$, **Figure 4C**; $p < 0.01$, **Figure 4D**). These results revealed that LINC00680 regulates the MAPK1 expression by sponging miR-320a in a ceRNA manner.

LINC00680 Inhibited Apoptosis and Promoted Proliferation by Sponging miR-320a in Jurkat Cells

Since MG is a T-cell-dependent autoimmune disorder, the proliferation and activation of T cells inevitably have a great influence on the occurrence and development of MG. The Jurkat cells were selected for the functional verification of MG (37). To detect whether LINC00680 could affect the proliferation and apoptosis of Jurkat cells by sponging miR-320a, we transfected Jurkat T cells with negative control, siLINC00680, and siLINC00680, along with a miR-320a inhibitor. Cell apoptosis ability was then detected by an Annexin V/PI assay. We found that the proportion of apoptosis increased significantly following the knockdown of LINC00680 in the Jurkat T-cell line. However, the addition of miR-320a inhibitors eliminated this trend (**Figures 5A,B**). CCK8 assays were selected to measure cell proliferation. Cell proliferation of the siLINC00680 group was lower than that of the control group; cotransfection with the miR-320a inhibitor reversed this effect (**Figure 5C**). These results revealed that LINC00680 promotes Jurkat T-cell proliferation and inhibits apoptosis by sponging miR-320a. Collectively, our results indicated that LINC00680 could regulate T-cell proliferation and apoptosis by sponging miR-320a and that this process is related to the immunological pathogenesis of MG.

DISCUSSION

Myasthenia gravis (MG) is an autoimmune disease that seriously threatens the health and even life of patients by causing muscle weakness. However, our ability to diagnose and treat MG remains limited. Therefore, it is vital for us to identify new biomarkers that can determine the severity

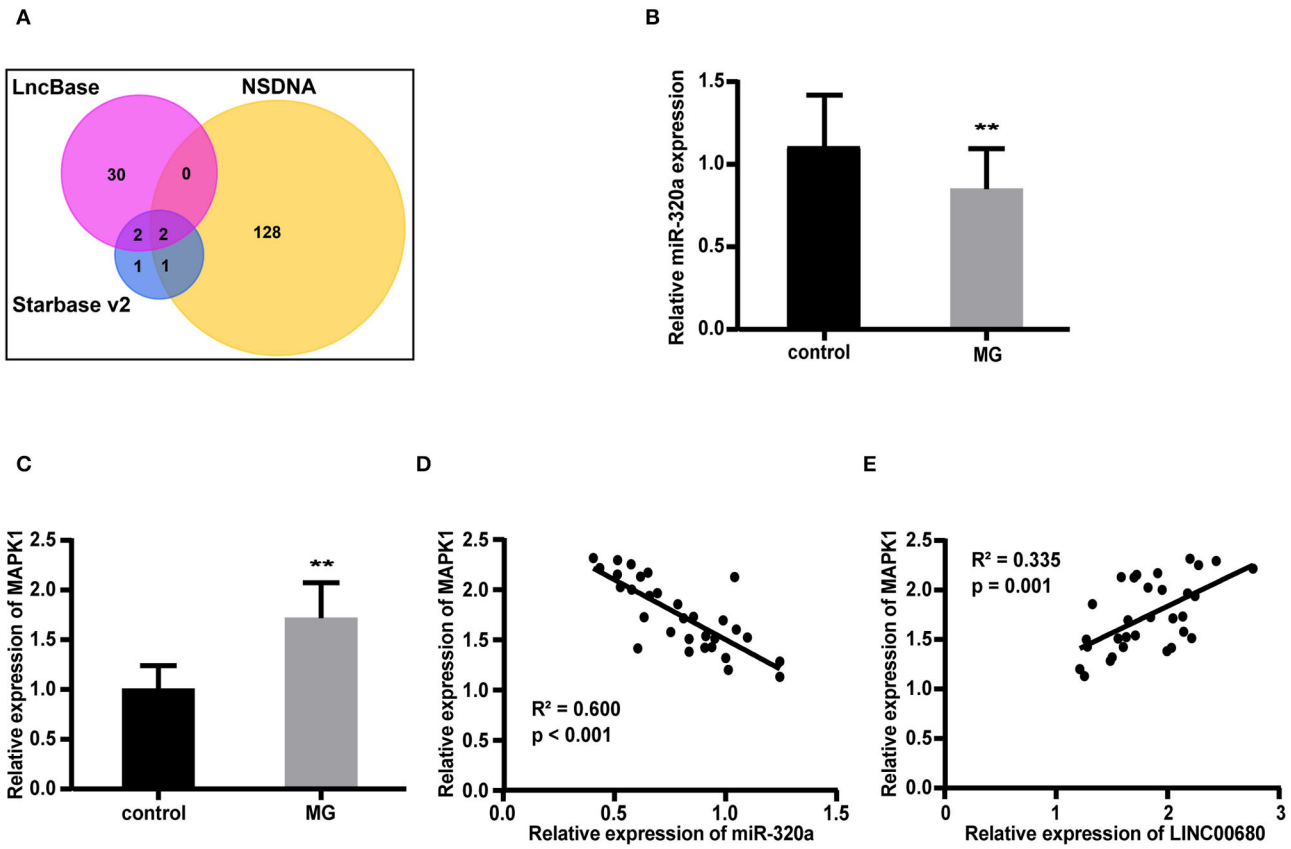


FIGURE 2 | Construction of a LINC00680-miR-320a-mitogen-activated protein kinase 1 (MAPK1) interaction network for MG. **(A)** Venn diagram showing the overlapping target genes of LINC00680 associated with MG that was predicted using the Starbase v2.0, DIANA-LncBase, and the Nervous System Disease NcRNAome Atlas (NSDNA) databases **(B,C)** miR-320a expression and MAPK1 messenger RNA (mRNA) expression were examined in 31 patients with MG and 31 control subjects by real-time PCR **(D)** Correlation analyses between MAPK1 messenger RNA (mRNA) expression and miR-320a expression in patients with MG **(E)** Correlation analyses between MAPK1 mRNA expression and LINC00680 expression in patients with MG (**$p < 0.01$).

of MG and serve as effective therapeutic targets. However, biomarkers that meet all the required criteria have yet to be identified. MG has a complex pathogenesis that involves genetic, immune, and environmental factors; collectively, these factors are strongly linked to the susceptibility and development of MG. In recent years, the involvement of lncRNA, as a ceRNA, in the occurrence and development of various diseases has attracted increasing levels of attention. For instance, the lncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1) has been shown to act as a ceRNA and regulates the IL-6 expression by sponging miR-1, thus affecting the severity of normal-tension glaucoma (38). These findings improved our understanding of the molecular mechanisms involved and provided a new perspective on how we might identify new biomarkers for MG.

In this study, we systematically detected the potential significance of LINC00680 in MG, which had never been investigated before. Our data displayed that the LINC00680 expression was observably upregulated in MG when compared with controls. When we further investigated the association between the LINC00680 expression levels and the MGC score and the QMG score and between EOMG and LOMG, we found that the LINC00680 expression levels were positively correlated with the MGC and QMG scores; no obvious difference was found between EOMG and LOMG.

Based on these results, we further explored the potential molecular mechanisms underlying the ability of LINC00680 to regulate MG. Bioinformatics analysis predicted that miR-320a may be the miRNA that targets LINC00680 in MG. As a member of the miR-320 family, miR-320a is located on human chromosome 8p21.3 and is closely related to disease progression, tumor invasion, and metastasis (39, 40). The expression of miR-320a was various in different diseases. For example, miR-320a was downregulated in cholangiocarcinoma (41), but it was upregulated in hepatocellular carcinoma (42). It has been reported that miR-320a expression was downregulated in patients with MG (1). Therefore, miR-320a was selected for further analysis. By reviewing the literature, we also found that MAPK1 is a target for miR-320a. Meanwhile, miR-320a is verified to regulate the secretion of MAPK1 in Jurkat cells (1). We also discovered that miR-320a overexpression reduced the

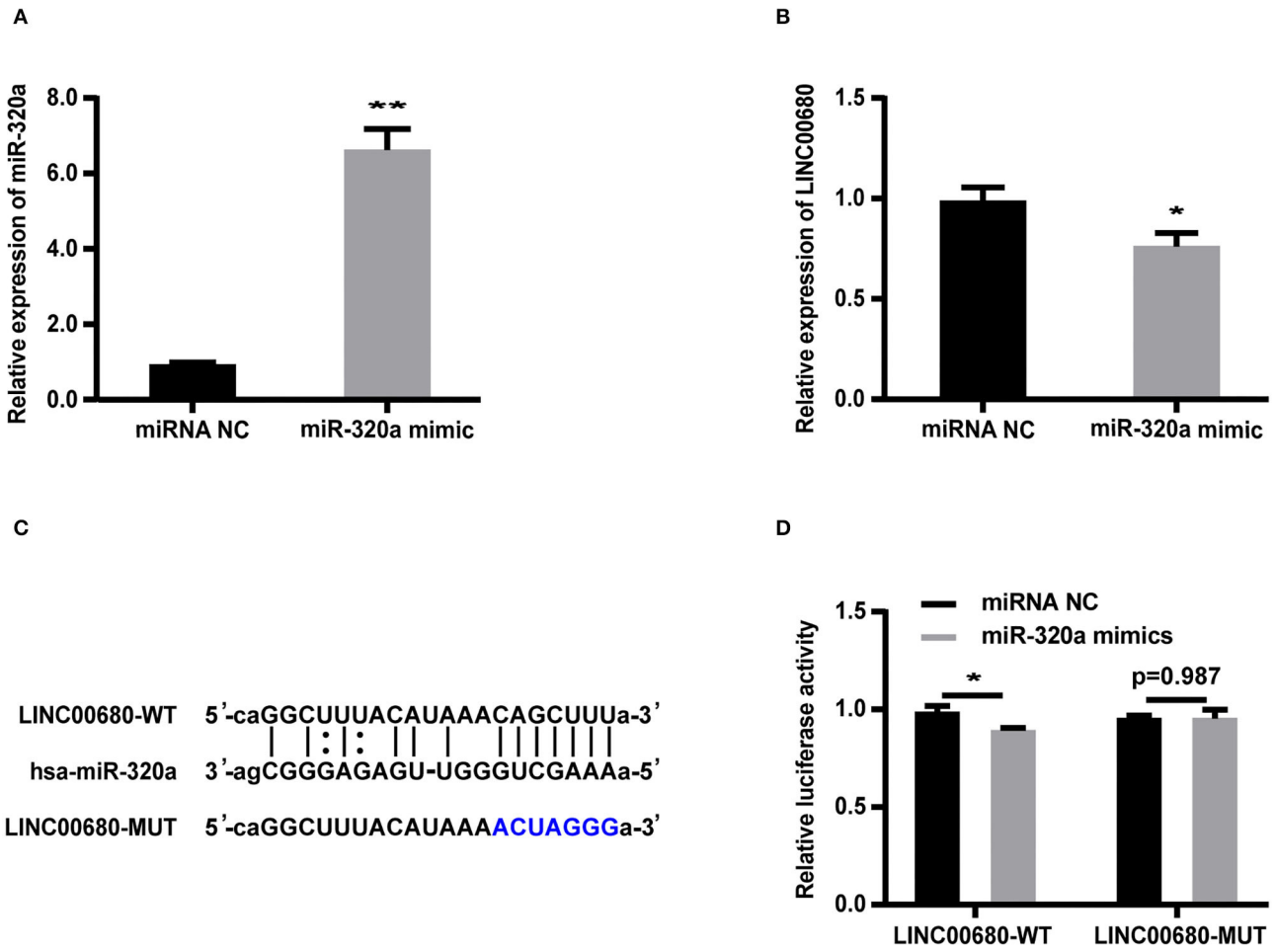


FIGURE 3 | LINC00680 is a target of miR-320a. **(A)** The transfection efficiency of the miR-320a mimic was detected by real-time PCR **(B)** The relative expression levels of LINC00680 transfected with miRNA NC or the miR-320a mimic in Jurkat cells for 48 h were detected by real-time PCR **(C)** The putative miR-320a binding sequence of the wild-type and mutated sequence of LINC00680 **(D)** A luciferase reporter plasmid containing LINC00680-WT or LINC00680-MUT was co-transfected with the miR-320a mimic or miRNA NC into HEK293T cells for 48 h. Luciferase activities were calculated as the ratio of Firefly/Renilla activities (*$p < 0.05$, **$p < 0.01$).

MAPK1 mRNA and protein expression levels in Jurkat cells. Then, we detected the miR-320a expression and the MAPK1 expression by qRT-PCR in patients with MG and normal controls. We detected a significant downregulation of miR-320a, along with a significant upregulation of MAPK1, in patients with MG. These discoveries were all consistent with the past publication (1). Moreover, we also found that there was a positive correlation between LINC00680 expression and MAPK1 expression. Therefore, we assumed that LINC00680 regulates the miR-320a/MAPK1 axis as a ceRNA to affect the severity of MG.

To further verify the existence of the LINC0680/miR-320a/MAPK1 axis, we carried out several experiments *in vitro*. First, we transfected miR-320a mimic into Jurkat cells and found that the transfection with miR-320a reduced the expression of LINC00680 in Jurkat cells. Then, luciferase reporter assays were selected to affirm that LINC00680 is a direct target of miR-320a. Finally, we transfected negative control, siLINC00680, and cotransfected siLINC00680 and miR-320a inhibitor into Jurkat cells, respectively. We found that the knockout of LINC00680 inhibited MAPK1 expression at both the protein and mRNA levels; however, these effects could be reversed when Jurkat cells were cotransfected with siLINC00680 and miR-320a inhibitor. In addition, cell proliferation ability and apoptosis ability analysis indicated that the knockdown of LINC00680 could not only inhibit cell proliferation but also promote cell apoptosis. However, these effects could also be reversed by the cotransfection of siLINC00680 and miR-320a inhibitors into Jurkat cells. These results indicated that lncRNA LINC00680 regulates MAPK1 through competitively binding miR-320a as a ceRNA. These findings increase our knowledge of the specific mechanisms underlying MG.

Mitogen-activated protein kinase 1 (MAPK1) is a vital member of the MAPK family. The activation of MAPK is closely related to the transcription and translation of cytokines

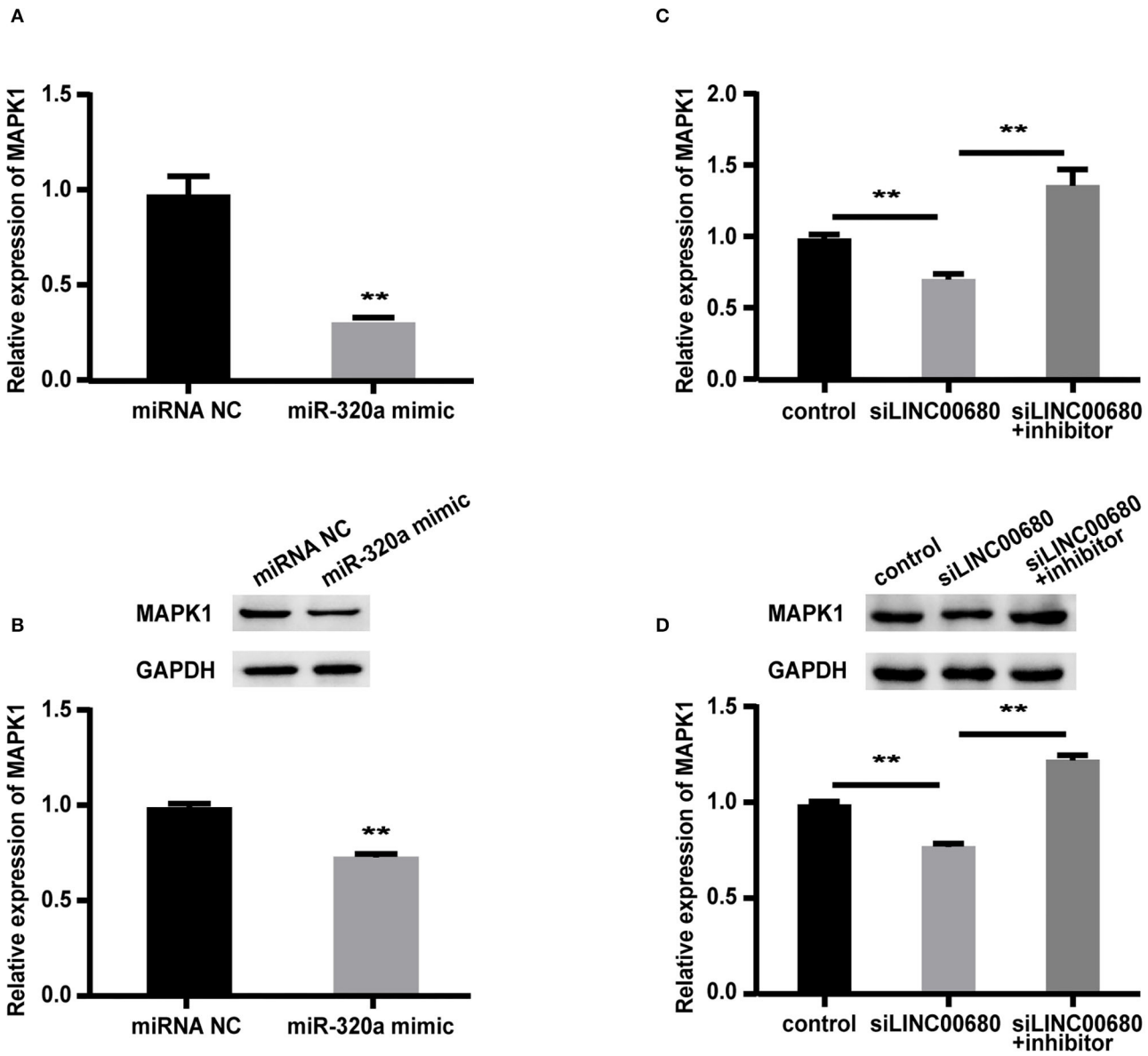


FIGURE 4 | LINC00680 regulated MAPK1 expression by binding miR-320a as a competing endogenous RNA (ceRNA). **(A)** The relative mRNA levels of MAPK1 were determined by real-time PCR after transfection with negative control or miR-320a mimic in Jurkat cells for 48 h **(B)** Relative protein expression levels of MAPK1 were determined by Western blotting after transfection with negative control or miR-320a mimic in Jurkat cells for 48 h **(C)** Relative mRNA levels of MAPK1 were determined by real-time PCR analysis after transfection with negative control, siLINC00680, and siLINC00680 + miR-320a inhibitor in Jurkat cells for 48 h **(D)** Relative protein expression levels of MAPK1 were determined by Western blotting after transfection with negative control, siLINC00680, and siLINC00680 + miR-320a inhibitor in Jurkat cells for 48 h (**$p < 0.01$).

(43). Furthermore, MAPK signaling pathways are essential for the synthesis and amplification of inflammatory factors (44). These inflammatory factors have been indicated to have a significant function in the immunological pathogenesis of MG (45). A study has shown that IL-2 could affect the pathogenesis of MG (46). It has also been reported that IFN-γ could affect the severity of experimental MG (14). A previous study has shown that miR-320a can repress the production of IFN-γ and IL-2 by directly inhibiting MAPK1 expression in MG (1). Therefore, these findings confirm that LINC00680 may affect the severity of MG by regulating the miR-320a/MAPK1 axis.

This is the first study to investigate the association between the LINC00680 expression and the severity of MG and, therefore, demonstrate the biological function and molecular mechanisms of LINC00680 in MG, which regulates the expression of MAPK1 by sponging miR-320a. However, several limitations to this study need to be addressed. First,

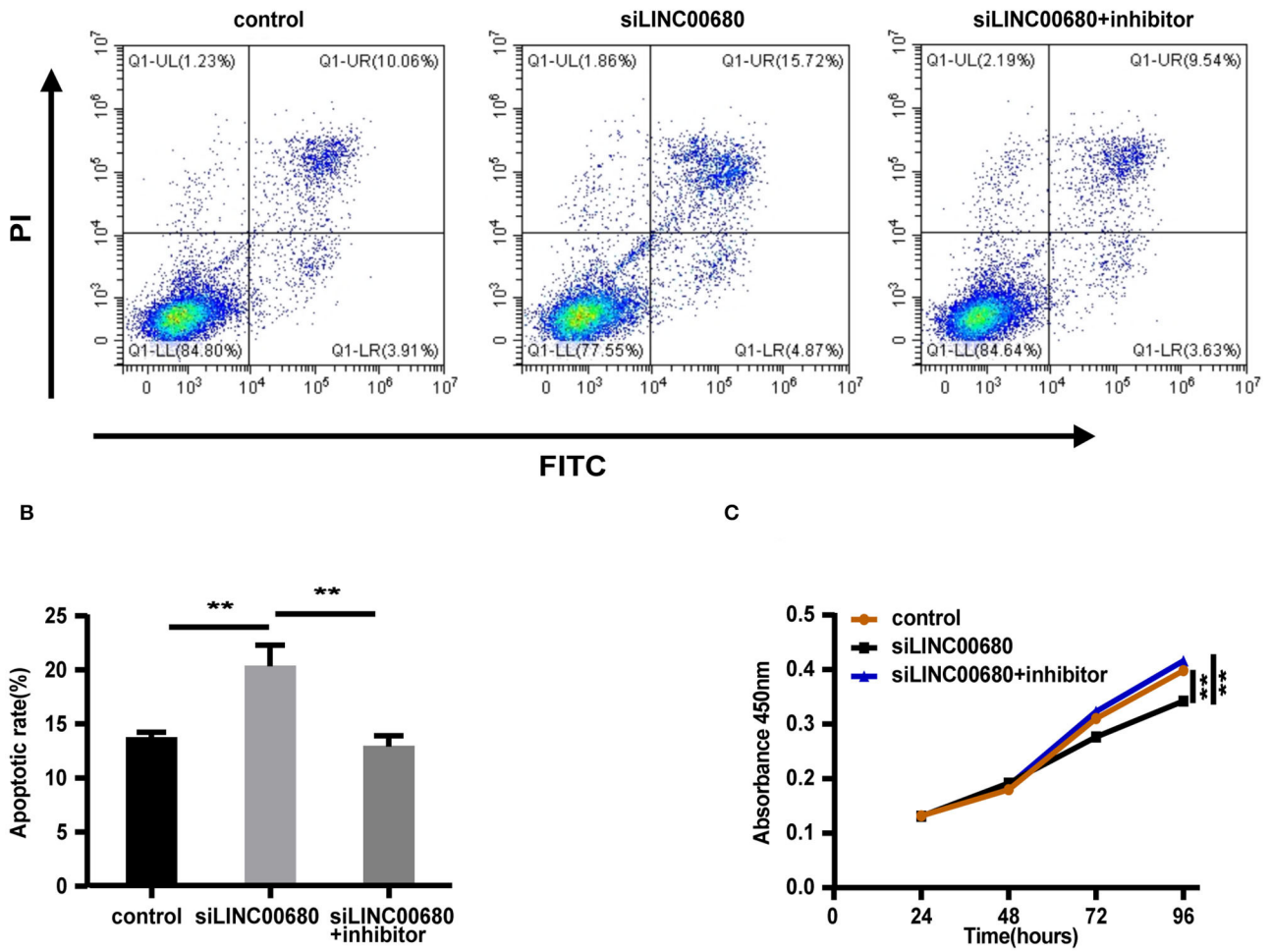


FIGURE 5 | LINC00680 inhibited apoptosis and promoted proliferation by sponging miR-320a. **(A)** After transfecting negative control, siLINC00680, or siLINC00680 + miR-320a inhibitor for 48 h, Jurkat cells were stained with Annexin-V-FITC/propidium iodide (PI) and apoptosis was detected by flow cytometric analysis **(B)** The apoptosis rate of Jurkat cells after transfecting negative control, siLINC00680, or siLINC00680 + miR-320a inhibitor for 48 h **(C)** Cell proliferation was analyzed by Cell Counting Kit-8 (CCK-8) assays after transfecting negative control, siLINC00680, or siLINC00680 + miR-320a inhibitor into Jurkat cells for 24, 48, 72, and 96 h (**$p < 0.01$).

the sample size of this study is not large enough and a larger sample size is needed to further confirm the results of this study. Second, more recovery experiments should be added to further confirm that LINC00680 sponges miR-320a affected T-cell proliferation and apoptosis by regulating MAPK1.

CONCLUSION

Our data demonstrated that LINC00680 may be associated with the severity of MG and act as a ceRNA. In this study, we identified LINC00680 as a new biomarker for the diagnosis, development, and treatment of MG.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of The Second Affiliated Hospital of Harbin Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LW and JW conceived and designed the study. LLiu performed the experiments, analyzed the data, and drafted the manuscript. HZ, XL, LLi, and TW provided intellectual support and contributed reagents and analytical tools for the study. LLi, QL, TW, ZC, and HG provided guidance on data analysis. SL, XW, SX, and TY revised the manuscript. All authors have read and approved the submitted version of the manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (Grant No: 81820108014, 82171396, 82071407, and 81901277), the National Key Research and Development Project (Grant No: 2018YFE0114400), and the Heilongjiang Provincial Natural Science Foundation (Grant No: LH2019H086 and YQ2021H012).

ACKNOWLEDGMENTS

The authors would like to thank all those who participated in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.833062/full#supplementary-material

REFERENCES

1. Cheng Z, Qiu S, Jiang L, Zhang A, Bao W, Liu P, et al. MiR-320a is downregulated in patients with myasthenia gravis and modulates inflammatory cytokines production by targeting mitogen-activated protein kinase 1. *J Clin Immunol.* (2013) 33:567–76. doi: 10.1007/s10875-012-9834-5
2. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis — autoantibody characteristics and their implications for therapy. *Nat Rev Neurol.* (2016) 12:259–68. doi: 10.1038/nrneurol.2016.44
3. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol.* (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3
4. Querol L, Illa I. Myasthenia gravis and the neuromuscular junction. *Curr Opin Neurol.* (2013) 26:459–65. doi: 10.1097/WCO.0b013e328364c079
5. Gilhus NE. Myasthenia gravis. *N Engl J Med.* (2016) 375:2570–25. doi: 10.1056/NEJMra1602678
6. Gilhus NE, Tzartos S, Evoli A, Palace J, Burns TM, Verschuuren J. Myasthenia gravis. *Nat Rev Dis Primers.* (2019) 5:3–19. doi: 10.1038/s41572-019-0079-y
7. Conti-Fine BM, Milani M, Kaminski HJ. Myasthenia gravis: past, present, and future. *J Clin Invest.* (2006) 116:2843–54. doi: 10.1172/JCI29894
8. Conti-Fine BM, Milani M, Wang W. CD4+T cells and cytokines in the pathogenesis of acquired myasthenia gravis. *Ann N Y Acad Sci.* (2008) 1132:193–209. doi: 10.1196/annals.1405.042
9. Poea-Guyon S, Christadoss P, Le Panse R, Guyon T, De Baets M, Wakkach A, et al. Effects of cytokines on acetylcholine receptor expression: implications for myasthenia gravis. *J Immunol.* (2005) 174:5941–9. doi: 10.4049/jimmunol.174.10.5941
10. Berrih-Aknin S, Le Panse R. Myasthenia gravis: a comprehensive review of immune dysregulation and etiological mechanisms. *J Autoimmun.* (2014) 52:90–100. doi: 10.1016/j.jaut.2013.12.011
11. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature.* (1996) 383:787–93. doi: 10.1038/383787a0
12. Carr AS, Cardwell CR, McCarron PO, McConville J. A systematic review of population based epidemiological studies in myasthenia gravis. *BMC Neurol.* (2010) 10:46. doi: 10.1186/1471-2377-10-46
13. Aguirre F, Manin A, Fernandez VC, Justo ME, Leoni J, Paz ML, et al. C3, C5a and anti-acetylcholine receptor antibody as severity biomarkers in myasthenia gravis. *Ther Adv Neurol Disord.* (2020) 13:1–8. doi: 10.1177/1756286420935697
14. Stegall T, Krolick KA. Myocytes respond to both interleukin-4 and interferon-gamma: cytokine responsiveness with the potential to influence the severity and course of experimental myasthenia gravis. *Clin Immunol.* (2000) 94:133–9. doi: 10.1006/clim.1999.4822
15. Petersson M, Feresiadou A, Jons D, Ilinca A, Lundin F, Johansson R, et al. Patient-Reported symptom severity in a nationwide myasthenia gravis cohort: cross-sectional analysis of the Swedish GEMG study. *Neurology.* (2021) 97:e1382–91. doi: 10.1212/WNL.0000000000012604
16. Lu M, Tian H, Cao YX, He X, Chen L, Song X, et al. Downregulation of miR-320a/383-sponge-like long non-coding RNA NLC1-C (narcolepsy candidate-region 1 genes) is associated with male infertility and promotes testicular embryonal carcinoma cell proliferation. *Cell Death Dis.* (2015) 6:e1960–73. doi: 10.1038/cddis.2015.267
17. Necsulea A, Soumillon M, Warnefors M, Liechti A, Daish T, Zeller U, et al. The evolution of lncRNA repertoires and expression patterns in tetrapods. *Nature.* (2014) 505:635–40. doi: 10.1038/nature12943
18. Wei MM, Zhou GB. Long non-coding RNAs and their roles in non-small-cell lung cancer. *Genomics Proteomics Bioinformatics.* (2016) 14:280–8. doi: 10.1016/j.gpb.2016.03.007
19. Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. *Canc Cell.* (2016) 29:452–63. doi: 10.1016/j.ccell.2016.03.010
20. Ginn L, Shi L, Montagna M, Garofalo M, et al. LncRNAs in non-small-cell lung cancer. *Noncoding RNA.* (2020) 6:25. doi: 10.3390/ncrna6030025
21. Senousy MA, Shaker OG, Sayed NH, Fathy N, Kortam MA. LncRNA GAS5 and miR-137 polymorphisms and expression are associated with multiple sclerosis risk: mechanistic insights and potential clinical impact. *ACS Chem Neurosci.* (2020) 11:1651–60. doi: 10.1021/acschemneuro.0c00150
22. Li Y, Zhang S, Zhang C, Wang M. LncRNA MEG3 inhibits the inflammatory response of ankylosing spondylitis by targeting miR-146a. *Mol Cell Biochem.* (2020) 466:17–24. doi: 10.1007/s11010-019-03681-x
23. Liu CH, Lu YL, Huang HT, Wang CF, Luo HC, Wei GJ, et al. Association of LncRNA-GAS5 gene polymorphisms and PBMC LncRNA-GAS5 level with risk of systemic lupus erythematosus in Chinese population. *J Cell Mol Med.* (2021) 25:3548–59. doi: 10.1111/jcmm.16438
24. Luo M, Liu X, Meng H, Xu L, Li Y, Li Z, et al. IFNA-AS1 regulates CD4+ T cell activation in myasthenia gravis though HLA-DRB1. *Clin Immunol.* (2017) 183:121–31. doi: 10.1016/j.clim.2017.08.008
25. Hu B, Niu L, Jiang Z, Xu S, Hu Y, Cao K. LncRNA XLOC_003810 promotes T cell activation and inhibits PD-1/PD-L1 expression in patients with myasthenia gravis-related thymoma. *Scand J Immunol.* (2020) 92:e12886. doi: 10.1111/sji.12886
26. Zhang XZ, Liu H, Chen SR. Mechanisms of long non-coding RNAs in cancers and their dynamic regulations. *Cancers (Basel).* (2020) 12:1245–65. doi: 10.3390/cancers12051245
27. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. et al. A ceRNA hypothesis: the rosetta stone of a hidden RNA language? *Cell.* (2011) 146:353–8. doi: 10.1016/j.cell.2011.07.014
28. Thomson DW, Dinger ME. Endogenous microRNA sponges: evidence and controversy. *Nat Rev Genet.* (2016) 17:272–83. doi: 10.1038/nrg.2016.20
29. Pan J, Fang S, Tian H, Zhou C, Zhao X, Tian H, et al. LncRNA JPX/miR-33a-5p/Twist1 axis regulates tumorigenesis and metastasis of lung cancer by activating Wnt/beta-catenin signaling. *Mol Cancer.* (2020) 19:9–25. doi: 10.1186/s12943-020-1133-9

30. Liang Y, Song X, Li Y, Chen B, Zhao W, Wang L, et al. LncRNA BCRT1 promotes breast cancer progression by targeting miR-1303/PTBP3 axis. *Mol Cancer*. (2020) 19:85–104. doi: 10.1186/s12943-020-01206-5
31. Zhang T, Tan P, Wang L, Jin N, Li Y, Zhang L, et al. RNALocate: a resource for RNA subcellular localizations. *Nucleic Acids Res*. (2017) 45:D135–8.
32. Shu G, Su H, Wang Z, Lai S, Wang Y, Liu X, et al. LINC00680 enhances hepatocellular carcinoma stemness behavior and chemoresistance by sponging miR-568 to upregulate AKT3. *J Exp Clin Cancer Res*. (2021) 40:45. doi: 10.1186/s13046-021-01854-5
33. Wang H, Feng L, Zheng Y, Li W, Liu L, Xie S, et al. LINC00680 promotes the progression of non-small cell lung cancer and functions as a sponge of miR-410-3p to enhance HMGB1 expression. *Onco Targets Ther*. (2020) 13:8183–96. doi: 10.2147/OTT.S259232
34. Li JH, Liu S, Zhou H, Qu LH, Yang JH. StarBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA, and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res*. (2014) 42:D92–7. doi: 10.1093/nar/gkt1248
35. Paraskevopoulou MD, Georgakilas G, Kostoulas N, Reczko M, Maragkakis M, Dalamagas TM, et al. DIANA-LncBase: experimentally verified and computationally predicted microRNA targets on long noncoding RNAs. *Nucleic Acids Res*. (2013) 41:D239–45. doi: 10.1093/nar/gks1246
36. Wang J, Cao Y, Zhang H, Wang T, Tian Q, Lu X, et al. NSDNA: a manually curated database of experimentally supported ncRNAs associated with nervous system diseases. *Nucleic Acids Res*. (2017) 45:D902–7. doi: 10.1093/nar/gkw1038
37. Kong X, Wang J, Cao Y, Zhang H, Lu X, Wang Y, et al. The long noncoding RNA MALAT-1 functions as a competing endogenous RNA to regulate MSL2 expression by sponging miR-338-3p in myasthenia gravis. *J Cell Biochem*. (2019) 120:5542–50. doi: 10.1002/jcb.27838
38. Yue JL, Zheng SF. Analysis of association between MALAT1 haplotype and the severity of normal-tension glaucoma (NTG). *J Cell Mol Med*. (2021) 25:9918–26. doi: 10.1111/jcmm.15906
39. Huang S, Zhu X, Ke Y, Xiao D, Liang C, Chen J, et al. LncRNA FTX inhibition restrains osteosarcoma proliferation and migration via modulating miR-320a/TXNRD1. *Cancer Biol Therapy*. (2020) 21:379–88. doi: 10.1080/15384047.2019.1702405
40. Prado MSG, Jesus ML, Goes TC, Mendonça LSO, Kaneto CM. Downregulation of circulating miR-320a and target gene prediction in patients with diabetic retinopathy. *BMC Res Notes*. (2020) 13:155–61. doi: 10.1186/s13104-020-05001-9
41. Zhu H, Zhai B, He C, Li Z, Gao H, Niu Z, et al. LncRNA TTN-AS1 promotes the progression of cholangiocarcinoma via the miR-320a/neuropilin-1 axis. *Cell Death Dis*. (2020) 11:637–47. doi: 10.1038/s41419-020-02896-x
42. Wen Y, Han J, Chen J, Dong J, Xia Y, Liu J, et al. Plasma miRNAs as early biomarkers for detecting hepatocellular carcinoma. *Int J Cancer*. (2015) 137:1679–90. doi: 10.1002/ijc.29544
43. Kaminska B, MAPK. signalling pathways as molecular targets for anti-inflammatory therapy–from molecular mechanisms to therapeutic benefits. *Biochim Biophys Acta*. (2005) 1754:253–62. doi: 10.1016/j.bbapap.2005.08.017
44. Carter AB, Monick MM, Hunninghake GW. Both Erk and p38 kinases are necessary for cytokine gene transcription. *Am J Respir Cell Mol Biol*. (1999) 20:751–8. doi: 10.1165/ajrcmb.20.4.3420
45. Yoshikawa H, Satoh K, Yasukawa Y, Yamada M. Cytokine secretion by peripheral blood mononuclear cells in myasthenia gravis. *J Clin Neurosci*. (2002) 9:133–6. doi: 10.1054/jocn.2001.1028
46. Liu X, Luo M, Meng H, Zeng Q, Xu L, Hu B, et al. MiR-181a regulates CD4 T cell activation and differentiation by targeting IL-2 in the pathogenesis of myasthenia gravis. *Eur J Immunol*. (2019). doi: 10.1002/eji.201848007

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership