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Plasma Myokine Profiles in Patients With AChR- and MuSK-Ab-Positive Myasthenia Gravis

Jo Woon Seok[a*]
Jinny Lee[b*]
MinGi Kim[c]
Min Ju Kim[c]
Ha Young Shin[c]
Seung Woo Kim[c]

[a]Mo-Im Kim Nursing Research Institute, College of Nursing, Yonsei University, Seoul, Korea
[b]Yonsei University College of Medicine, Seoul, Korea
[c]Department of Neurology, Yonsei University College of Medicine, Seoul, Korea

Background and Purpose Myokines include cytokines secreted by muscle fibers, which are the final targets of myasthenia gravis (MG). This pilot study investigated whether myokine plasma concentrations are altered in patients with MG and assessed the association between the concentration of each myokine and disease severity.

Methods We compared the plasma concentrations of 15 myokines in 63 patients with acetylcholine receptor antibody (Ab)-positive MG and 14 with muscle-specific tyrosine kinase Ab-positive MG (MuSK MG) with those in 15 healthy controls. Plasma myokine concentrations were measured using a Luminex multiplex assay kit with magnetic beads that contained Abs for 15 myokines. Correlations between myokine concentration and clinical scale results were analyzed.

Results The concentration of fractalkine in plasma was higher in MG (median [interquartile range]=419.6 [38.7−732.5] pg/mL) than in controls (158.5 [0.0−313.2] pg/mL, p=0.034). The leukemia inhibitory factor concentration was also found to be higher in MuSK MG (29.9 [8.7−40.1] pg/mL) than in healthy controls (7.6 [0.0−15.6] pg/mL, p=0.013). Fatty-acid-binding protein 3 (FABP3) concentrations in plasma were positively associated with clinical parameters for MG severity, including scores on the Quantitative Myasthenia Gravis score (p=0.008), Myasthenia Gravis Activities of Daily Living (p=0.003), and Myasthenia Gravis Composite (p=0.024) scales. FABP3 concentration in plasma tended to decrease after treatment in patients without additional relapse but increased in those with further relapse.

Conclusions The plasma myokine profile was significantly altered in patients with MG. FABP3 concentration may be useful in assessing disease severity and predicting the treatment response.

Keywords myasthenia gravis; myokine; cytokine.

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune neuromuscular disease clinically characterized by fluctuating skeletal muscle weakness. The autoimmune target protein is located in the muscle membrane in most cases; the muscle can therefore be considered the final target. However, few studies have considered the state and role of skeletal muscle in MG pathophysiology. Previous studies found that cytokine and chemokine expression levels were increased in the skeletal muscles of experimental autoimmune MG and in patients with MG.[1-3] There is recent evidence that transcriptomic changes related to cellular metabolism are present in the muscle cells of patients with MG.[4] Caveolin-3, a membrane protein that is expressed in muscle cells and is involved in muscle repair,[5] is strongly expressed in the muscle tissues of patients with MG.[6] Elevated endoplasmic reticulum stress and muscle regeneration impairment have also been reported in the skeletal muscles of patients with MG.[7,8] These find-

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Correspondence
Seung Woo Kim, MD
Department of Neurology, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel +82-2-2228-1600
Fax +82-2-393-0705
E-mail kswneuro@yuhs.ac

*These authors contributed equally to this work.

ings indicate that the muscle fiber itself is profoundly affected and pathologically altered in MG.

Myokines are cytokines and other small proteins that are synthesized in and produced by muscle fibers in response to muscle contraction.[9] The expression levels of certain myokines increase after exercise, such as interleukin (IL)-6, IL-8, leukocyte-inducing factor, connective tissue growth factor, fatty-acid-binding protein 3 (FABP3), and osteonectin.[10-13] An association has therefore been proposed between myokines and various skeletal muscle diseases. The expression of follistatin-like protein 1 (FSTL-1), which stimulates angiogenesis and vascularization in muscles, is decreased in cancer-associated cachexia.[14] Altered myokine levels have also been observed in inflammatory myopathies[15] and muscular dystrophies.[16] The findings of those studies suggest that myokine level is significantly influenced by the functional status of muscles in related diseases. Based on these associations, myokines are being considered as potential targets for personalized treatment[17] and as biomarkers for disease severity.[18]

This pilot study investigated whether myokine concentrations in plasma are altered in patients with MG compared with healthy controls. We also compared the myokine concentrations in plasma of patients with acetylcholine receptor antibody (Ab)-positive MG (AChR MG) and with muscle-specific tyrosine kinase Ab-positive MG (MuSK MG) with those in healthy controls. We further analyzed the associations between myokine concentrations and the clinical variables associated with MG.

METHODS

Sample acquisition

We analyzed the plasma samples of 77 adult patients diagnosed with MG at Severance Hospital between January 2019 and June 2021. The MG diagnoses were based on clinical symptoms, a positive response to the neostigmine test, decrement response after repetitive nerve stimulation, and the presence of AChR and/or MuSK antibodies. There were 63 and 14 samples obtained from patients with AChR MG and with MuSK MG, respectively. The plasma samples of 19 patients with MG were collected at two time points: during exacerbation of MG and after treatment. The controls comprised 15 samples that were obtained from a healthy population with no evidence of disease.

This study was approved by the Institutional Review Board of Severance Hospital (IRB No. 4-2021-1417, 4-2020-0869, 4-2019-0471). Informed consent was obtained from all subjects, and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Data collection

The demographic and clinical data of patients at the time of sampling were recorded retrospectively, including the age at sampling, sex, age at MG onset, type of MG, Myasthenia Gravis Foundation of America (MGFA) classifications at nadir and at the time of sampling, presence of thymoma, thymectomy findings, AChR Ab level, and treatment type. The MGFA clinical classification is based on the type of muscle groups predominantly involved and weakness severity, which ranges from class I (ocular muscle weakness only) to class V (state of intubation).[19] MG severity at the time of sampling was also assessed using the Quantitative Myasthenia Gravis (QMG), Myasthenia Gravis Activities of Daily Living (MG ADL), and MG Composite scales. The QMG is a standardized quantitative scoring system developed to objectively evaluate MG severity.[20] It consists of 13 items with a total score of 0−39, where a higher score indicates more-severe disease. The MG ADL is a symptom-based eight-question survey that was developed to assess MG severity. The total score ranges from 0 to 24, where a higher score indicates more-severe disease.[21] The MG Composite scale consists of ten items that measure the symptoms and signs of MG, each being weighted based on symptom severity. The total score ranges from 0 to 50, where a higher score indicates more-severe disease.[22]

Myokine measurements

The plasma myokine concentrations were measured using a Luminex multiplex assay kit with magnetic beads (HMYOMAG-56K MILLIPLEX MAP Human Myokine Magnetic Bead Panel, MilliporeSigma, Oakville, ON, Canada). The beads were conjugated with the antibodies for the following 15 myokines identified in previous studies: apelin, brain-derived neurotrophic factor (BDNF), erythropoietin, FABP3, fibroblast growth factor 21 (FGF21), fractalkine, FSTL-1, IL-6, IL-15, irisin, leukemia inhibitory factor (LIF), myostatin, oncostatin, osteocrin, and osteonectin. Briefly, the beads were incubated using diluted plasma from patients with MG or healthy controls, washed with a buffer, and measured on a Luminex 100 machine (Luminex, Austin, TX, USA). The results of the Luminex multiplex assay were obtained as median fluorescence intensity (MFI) values, and the concentration of each myokine was calculated from the MFI using the MasterPlex QT 2010 device (Hitachi, San Bruno, CA, USA). Plasma myokine concentrations were measured in duplicate, for which the intra-assay coefficients of variation (CVs) ranged from 2.9% to 19.2%: the CVs of BDNF, erythropoietin, FABP3, FGF21, fractalkine, IL-6, IL-15, irisin, osteocrin, and osteonectin were <10%, while those for apelin, FSTL-1, LIF, myostatin, and oncostatin were ≥10%.

Statistical analyses

Data are expressed as number (percentage), mean±standard deviation, or median [interquartile range] values. The chi-square test and *t*-test were used to compare categorical and continuous variables between the two study groups, respectively. Comparisons of plasma myokine concentrations among the three groups were conducted using the Kruskal–Wallis test followed by Dunn's multiple-comparisons test, and the alpha level was adjusted to 0.017 (0.05/3 groups ≈0.017). Linear regression analysis was used to assess the correlations between myokine concentrations and other clinical variables. Myokine concentrations and clinical parameters before and after treatment were compared using the Wilcoxon signed-rank test. Statistical analyses were conducted using SPSS 26.0 (IBM Corp., Armonk, NY, USA), with significance set at $p<0.05$.

RESULTS

Clinical characteristics

Plasma samples obtained from 77 patients with MG and 15 healthy controls were analyzed. The age of the 77 patients with MG was 51.1±15.8 years, and 46 of them (59.7%) were female. Of the 77 samples from patients with MG, 29 were collected during exacerbation of MG, 13 during remission, and 35 when the patients were symptomatic but clinically stable. The age of the 15 healthy controls was 46.3±13.8 years, and 7 of them (46.7%) were female.

Table 1 lists the detailed clinical features of the 63 patients with AChR MG and 14 with MuSK MG. The ages of the patients at the time of sampling were 50.1±16.2 and 55.3±13.9 years in the AChR MG and MuSK MG groups, respectively. The proportion of females was higher in patients with MuSK MG (92.9%) than in those with AChR MG (52.4%, $p=0.005$). There were no differences among the subtype classifications of MG or the MGFA clinical classifications at nadir. The proportion of patients who underwent thymectomy was higher in the AChR MG group (73.0%) than in the MuSK MG group (7.1%, $p<0.001$).

Comparison of myokine concentrations between patients with MG and healthy controls

Plasma myokine concentrations were compared among the entire MG cohort ($n=77$; i.e., including patients with both AChR MG and MuSK MG) and healthy controls ($n=15$). The plasma fractalkine concentration was significantly higher in patients with MG (419.6 [38.7–732.5] pg/mL) than in healthy controls (158.5 [0.0–313.2] pg/mL, $p=0.034$). However, no significant differences were observed in the concentrations of the remaining myokines between the MG and control groups.

Table 1. Demographic and clinical features of patients with AChR Ab-positive MG and MuSK Ab-positive MG

	AChR MG ($n=63$)	MuSK MG ($n=14$)	*p*
Age, years	50.1±16.2	55.3±13.9	0.276
Sex, female	33 (52.4)	13 (92.9)	0.005
Age at onset, years	41.5±17.6	44.6±15.1	0.533
Subtype classification of MG			0.198
Ocular MG	9 (14.3)	0 (0)	
Generalized MG	54 (85.7)	14 (100)	
AChR Ab titer at diagnosis, nmol/L	10.4±5.6		
MGFA classification at nadir			0.121
I	9 (14.3)	0 (0)	
II	18 (28.6)	7 (50.0)	
III	23 (36.5)	2 (14.3)	
IV	2 (3.2)	1 (7.1)	
V	11 (17.5)	4 (28.6)	
Thymectomy	46 (73.0)	1 (7.1)	<0.001
Thymic pathology*			0.064
Normal	3 (6.5)	0 (0)	
Thymic hyperplasia	12 (26.1)	0 (0)	
Thymoma	29 (63.0)	0 (0)	
Thymic cyst	1 (2.2)	0 (0)	
Unknown	1 (2.2)	1 (100)	
Treatment, overall			
Prednisolone	46 (73.0)	14 (100)	0.031
Azathioprine	22 (34.9)	4 (28.6)	0.762
Cyclosporine	5 (7.9)	3 (21.4)	0.154
Tacrolimus	21 (33.3)	6 (42.9)	0.545
Mycophenolate mofetil	6 (9.5)	2 (14.3)	0.632

Data are mean±SD or number (%) values.
*Results for 47 patients who underwent thymectomy.
Ab, antibody; AChR, acetylcholine receptor; MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; MuSK, muscle-specific tyrosine kinase.

Comparison of myokine concentrations among patients with AChR MG, patients with MuSK MG, and healthy controls

We compared myokine concentrations in plasma among the AChR MG, MuSK MG, and control groups (Table 2). Plasma concentrations of apelin ($p=0.026$), LIF ($p=0.039$), myostatin ($p=0.009$), and osteocrin ($p=0.030$) differed significantly among the three groups. There were no significant differences in the plasma concentrations of other myokines. The post hoc analysis indicated that the LIF concentration was significantly higher in patients with MuSK MG (29.9 [8.7–40.1] pg/mL) than in healthy controls (7.6 [0.0–15.6] pg/mL, $p=0.013$). In contrast, plasma concentrations of apelin, myostatin, and osteocrin did not differ significantly.

Associations between myokine concentrations and clinical variables in patients with MG

Patients with MG were classified into groups based on sex, history of diabetes mellitus, MG subtype (ocular vs. generalized), current prednisolone treatment, and history of thymectomy; plasma myokine concentrations were compared among these groups. There were no significant differences between the groups classified by sex, MG subtype, or current prednisolone treatment. Myokine concentrations did not differ between the patients with and without diabetes mellitus, with the exception of FABP3, which was higher in those with diabetes mellitus (2,020.8 [1,452.8–4,014.6] pg/mL vs. 1,250.5 [824.4–1,646.5] pg/mL, p=0.002). Concentrations of erythropoietin (p=0.006), FGF21 (p=0.001), fractalkine (p=0.008), LIF (p=0.022), myostatin (p=0.007), oncostatin (p=0.016), and osteocrin (p=0.013) were significantly lower in patients with MG who underwent thymectomy (Fig. 1).

The associations of each myokine concentration with age and disease duration were also analyzed. The FABP3 concentration was positively correlated with age (β=0.272, p=0.009), whereas the BDNF concentration was negatively correlated with age (β=-0.234, p=0.025). The plasma concentrations of FGF21 (β=-0.225, p=0.049), IL-6 (β=-0.246, p=0.031), myostatin (β=-0.247, p=0.030), and oncostatin (β=-0.227, p=0.047) were negatively associated with the disease duration of MG.

Table 2. Comparison of myokine levels in plasma among patients with AChR MG, patients with MuSK MG, and healthy controls

	Detection rate (%)	AChR MG (n=63)	MuSK MG (n=14)	Healthy controls (n=15)	p
Apelin	25.0	0 [0.0–72.1]	61.4 [0.0–187.8]	0 [0.0–72.1]	0.026
BDNF	100	754.2 [407.8–1,515.6]	780.1 [484.4–1,910.7]	913.7 [523.1–4,141.4]	0.428
Erythropoietin	62.0	1,386.8 [0.0–3,859.6]	3,635.3 [0.0–5,657.1]	738.2 [0.0–2,643.4]	0.074
FABP3	100	1,283.8 [863.0–1,975.3]	1,524.3 [1,053.3–2,315.1]	1,064.2 [847.4–1,634.9]	0.257
FGF21	21.7	0 [0.0–0.0]	0 [0.0–78.7]	0 [0.0–54.5]	0.107
Fractalkine	75.0	375.4 [0.0–657.2]	639.0 [166.8–945.9]	158.5 [0.0–313.2]	0.054
FSTL-1	58.7	380.1 [0.0–4,190.4]	3,044.1 [706.7–5,920.2]	1,298.1 [0.0–3,117.4]	0.247
IL-6	29.3	0 [0.0–1.7]	0 [0.0–2.0]	0 [0.0–9.8]	0.556
IL-15	28.3	0 [0.0–0.7]	0 [0.0–2.7]	0 [0.0–0.0]	0.614
Irisin	1.0	0 [0.0–0.0]	0 [0.0–0.0]	0 [0.0–0.0]	0.351
LIF	84.8	11.2 [3.1–25.9]	29.9 [8.7–40.1]	7.6 [0.0–15.6]	0.039*
Myostatin	19.6	0 [0.0–0.0]	30.2 [0.0–637.7]	0 [0.0–560.7]	0.009
Oncostatin	81.5	1.9 [0.4–3.3]	2.3 [1.7–3.9]	0.8 [0.0–7.8]	0.248
Osteocrin	97.8	114.2 [84.5–159.0]	180.3 [129.5–512.3]	114.2 [92.1–152.3]	0.030
Osteonectin	98.9	109.3 [77.4–138.9]	88.3 [69.2–111.5]	100.1 [77.4–192.5]	0.463

Data are median [interquartile range] values.
*Significant between MuSK MG and healthy controls.
AChR, acetylcholine receptor; BDNF, brain-derived neurotrophic factor; FABP3, fatty-acid-binding protein 3; FGF21, fibroblast growth factor 21; FSTL-1, follistatin-like protein 1; IL, interleukin; LIF, leukemia inhibitory factor; MG, myasthenia gravis; MuSK, muscle-specific tyrosine kinase.

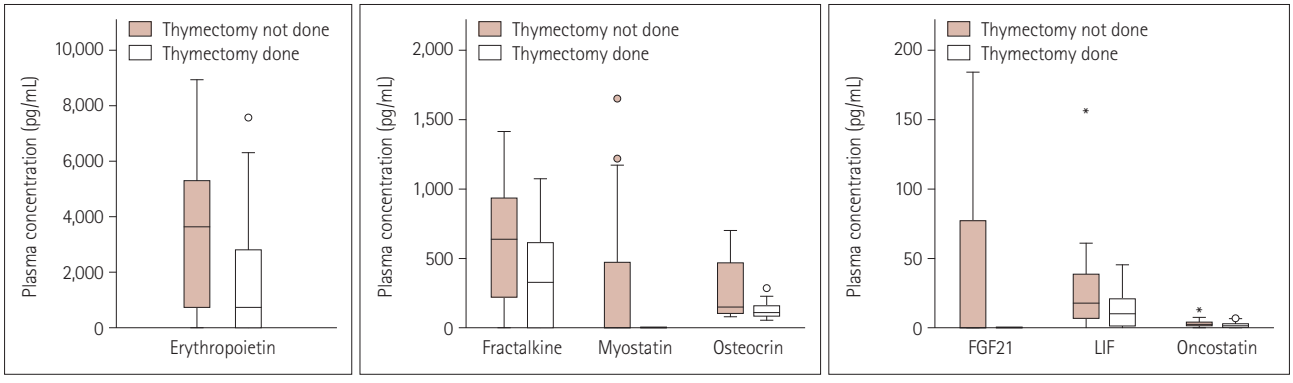

Fig. 1. Comparison of plasma myokine concentrations between patients with myasthenia gravis who did and did not undergo thymectomy. Concentrations of erythropoietin (p=0.006), FGF21 (p=0.001), fractalkine (p=0.008), LIF (p=0.022), myostatin (p=0.007), oncostatin (p=0.016), and osteocrin (p=0.013) were significantly lower in patients who had undergone thymectomy. Circles denote outliers and asterisks denote extreme outliers. FGF21, fibroblast growth factor 21; LIF, leukemia inhibitory factor.

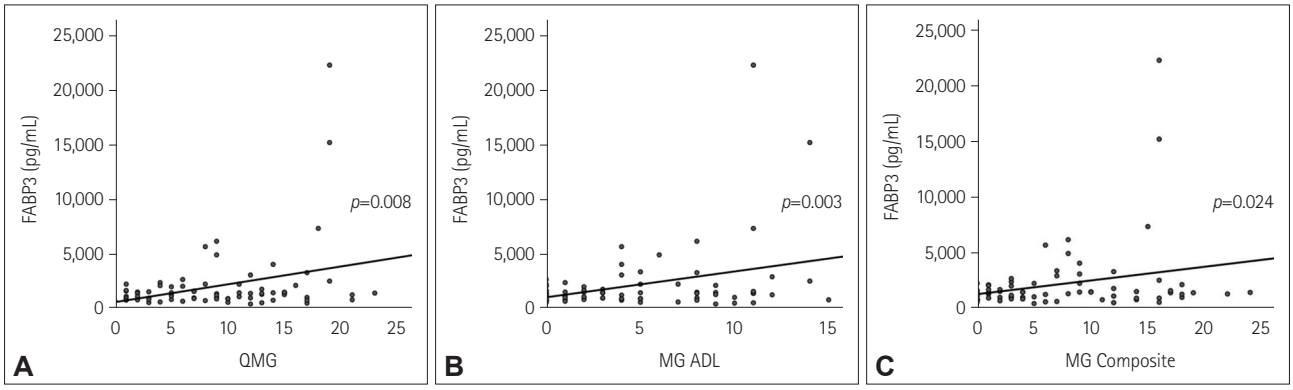

Fig. 2. Association between the FABP3 concentration in plasma and disease severity. Plasma FABP3 concentrations presented significant positive associations with scores on the (A) QMG, (B) MG ADL, and (C) MG Composite scales at the time of sampling. ADL, Activity of Daily Living; FABP3, fatty-acid-binding protein 3; MG, myasthenia gravis; QMG, Quantitative MG.

Associations between myokine concentrations and MG severity

We further analyzed whether certain types of myokines were associated with disease severity according to the scores on the QMG, MG ADL, and MG Composite scales. The plasma FABP3 concentration had a significant positive association with the scores on the QMG (β=0.310, p=0.008), MG ADL (β=0.331, p=0.003), and MG Composite (β=0.259, p=0.024) scales, indicating that higher FABP3 concentrations were associated with more-severe MG (Fig. 2). Since the FABP3 concentration was associated with age and diabetes mellitus, we analyzed the association between the FABP3 concentration and disease severity after adjusting for age and diabetes mellitus. The FABP3 concentration remained significantly associated with the scores on the QMG (p=0.015), MG ADL (p=0.004), and MG Composite (p=0.049) scales after adjusting for age and diabetes mellitus. None of the remaining myokine concentrations demonstrated significant associations with those scores. The FABP3 concentration also demonstrated a positive correlation with the MGFA classification at the time of sampling (Fig. 3).

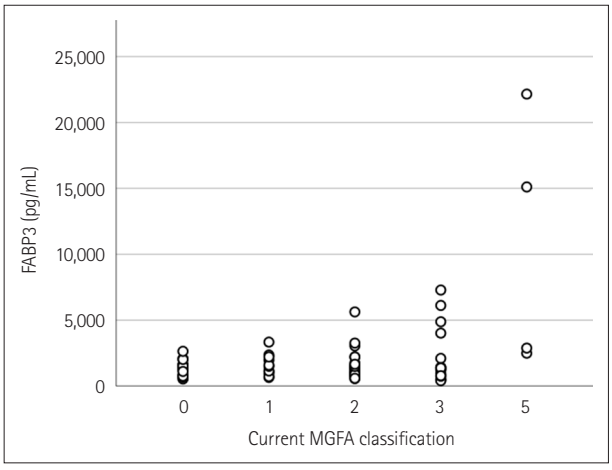

Fig. 3. Associations between FABP3 concentration in plasma and MGFA clinical classification at the time of sampling. FABP3 concentration tended to increase with the MGFA classification. FABP3, fatty-acid-binding protein 3; MGFA, Myasthenia Gravis Foundation of America.

Comparison of FABP3 concentrations before and after treatment

Changes in the FABP3 concentration were assessed in 19 patients with MG whose blood was sampled before and after treatment. The duration between the first and second sampling times was 253 (57–419) days. The score on the MG ADL scale decreased from 9.0 (5.0–11.0) to 1.0 (0.0–3.0) after treatment (p<0.001). Overall, there was no significant difference in the plasma FABP3 concentration between before (1,430.3 [956.5–2,883.0] pg/mL) and after (1,505.8 [1,140.5–3,214.5] pg/mL, p=0.872) treatment. Patients were classified into two groups based on the occurrence of additional exacerbation within 6 months from the time of the second sampling. Additional exacerbation occurred in ten patients (relapse group) and not in nine patients (nonrelapse group). Changes in the FABP3 concentration in the relapse and nonrelapse groups are shown in Fig. 4. Although not significant, the plasma FABP3 concentration decreased from 2,098.7 (1,346.3–5,310.7) pg/mL to 1,505.8 (1,305.9–3,580.0) pg/mL in the nonrelapse group (p=0.139). In contrast, the FABP3 concentration increased from 1,204.2 (552.7–1,728.5) pg/mL to 1,431.8 (1,136.1–2,579.9) pg/mL in the relapse group (p=0.047).

DISCUSSION

Significant differences in plasma myokines profiles were observed between the patients with MG and healthy controls. The fractalkine concentration in plasma was higher in patients with MG than in healthy controls, and the LIF concen-

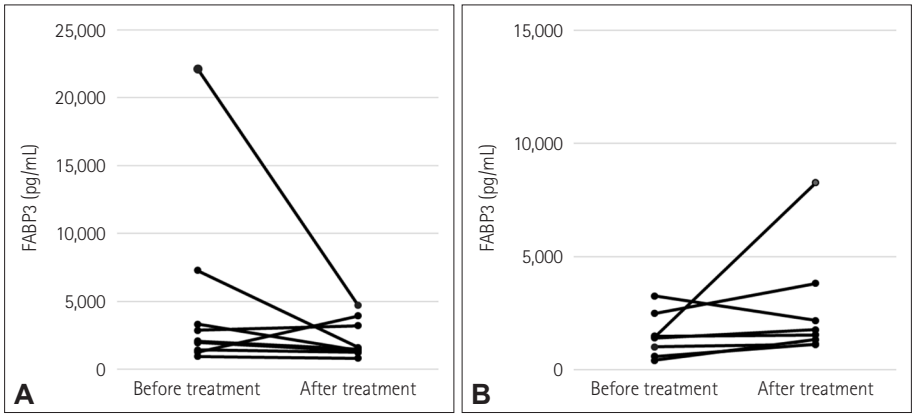

Fig. 4. Changes in FABP3 concentration in plasma before and after treatment in patients with myasthenia gravis. (A) FABP3 concentration tended to decrease after treatment in patients who did not experience additional relapse (p=0.139) and (B) increased in patients who did experience further relapse (p=0.047). FABP3, fatty-acid-binding protein 3.

tration was also higher in MuSK MG. The concentrations of certain myokines were also associated with the clinical variables of MG. The concentrations of fractalkine, FGF21, and myostatin were lower in patients who had undergone a thymectomy. The concentrations of myostatin and IL-6 were negatively correlated with the disease duration of MG. The concentration of FABP3 in plasma was found to have a positive association with disease severity according to the scores on the QMG, MG ADL, and MG Composite scales. Plasma myokine concentrations may provide clues to better understand the pathomechanism of MG and the changes that occur in muscle fibers during the disease course. Furthermore, the FABP3 concentration may be useful as a marker for assessing MG severity.

Overall, fractalkine concentration was increased in patients with MG compared with healthy controls. Fractalkine (CX3CL1/neurotactin) is a chemokine that exists in both membrane-bound and free-floating soluble forms, which promote cell adhesion and displays chemoattractant activity, respectively.[23] Increased fractalkine expression has been observed in various autoimmune diseases, including inflammatory myositis, systemic lupus erythematosus, and rheumatoid arthritis.[24,25] Fractalkine concentration in serum is correlated with the disease severities of inflammatory myositis and rheumatoid vasculitis,[25,26] and with the proinflammatory cytokine concentration in Sjogren's syndrome.[27] The expression of membrane-bound fractalkine is increased by inflammatory cytokines such as tumor necrosis factor-alpha, interferon (IFN)-gamma, and IL-1,[28,29] which are also elevated in the sera of some patients with MG.[30-32] The association between fractalkine concentration and MG severity is not as clear as that between fractalkine concentration and the severity of other autoimmune diseases; however, we hypothesize that the inflammatory process in MG contributes to the increased fractalkine concentration. However, caution is required when interpreting the source of fractalkine expression. Although fractalkine is known to be expressed in the human skeletal muscle,[33] it is mostly expressed in the infiltrated mononuclear cells and endothelial cells of patients with inflammatory myositis.[25] Further studies are therefore required to elucidate whether the increased fractalkine concentration in patients with MG is a muscular response to the disease or is merely a marker of systemic inflammation.

A higher FABP3 concentration was associated with older age and diabetes mellitus in the present study. FABP3 is a cytosolic lipid transport protein that regulates fatty acid metabolism and is mostly distributed in the skeletal muscles and cardiomyocytes.[34] FABP3 stimulates glucose uptake in muscle cells via the phosphorylation of AMP-activated protein kinase in response to insulin, thereby maintaining serum glucose homeostasis in vivo.[35] FABP3 expression was found to be associated with the development of obesity and increased serum insulin levels in an obese-mouse model.[35] Another experimental study found that FABP3 expression was increased more in the skeletal muscle of aged mice than in young mice.[36] Therefore, the associations identified in the present study among FABP3, older age, and diabetes mellitus were consistent with previous reports.

The FABP3 concentration remained significantly associated with MG severity after adjusting for age and diabetes mellitus. Previous studies have consistently demonstrated the role of FABP3 as a marker of skeletal muscle toxicity. FABP3 mediates fatty acid uptake in skeletal muscle and transports it to the mitochondrial β-oxidation system.[37] Physiologic or pathologic conditions with increased metabolic demand may increase FABP3 expression to result in its release into the peripheral blood. A rapid increase in the FABP3 concentration was observed after eccentric exercise in healthy participants.[38]

The FABP3 concentration in blood is increased in patients with peripheral artery disease, in whom decreased perfusion may induce decreased mitochondrial activity and increased oxidative stress.[39] Increased FABP3 expression was observed on immunohistochemistry staining in the skeletal muscles of patients with severe peripheral artery disease.[40] The FABP3 concentration is also significantly correlated with myositis severity. Zhang et al.[18] found that FABP3 expression was increased in the skeletal muscles of patients with idiopathic inflammatory myopathies and that the serum FABP3 concentration was correlated with muscle strength. Gupta et al.[41] similarly found that the serum FABP3 concentration was increased in patients with idiopathic inflammatory myopathies and was decreased through treatment. The association between FABP3 concentration and MG severity found in the present study was similar to that between FABP3 concentration and myositis. These findings imply that muscle toxicity may at least partially contribute to MG severity. This is worth noting as muscle weakness in MG is generally regarded to result from impaired neuromuscular transmission rather than directly from muscle toxicity. However, complement activation induced by AChR Ab and the formation of a membrane-attacking complex can cause localized myofiber damage around neuromuscular junctions,[42] which partially mimics the inflammatory process in myositis.[43]

The FABP3 concentration in patients with MG did not change significantly after treatment. This contrasts with previous observations of myositis, in which FABP3 significantly decreased in patients who responded to treatment.[41] This discrepancy could be due to different pathomechanisms underlying muscle weakness in the two diseases. The weakness is caused by direct damage to muscle fibers in myositis, and clinical improvement takes place following recovery from muscle damage. In contrast, the weakness mechanism is more diverse in MG than in myositis and includes the blockage of AChR, disruption of the postsynaptic membrane, endocytosis of AChR, and possibly direct muscle damage. The degree of muscle damage may therefore not always be correlated with clinical symptoms. However, patterns of changes in the FABP3 concentration after treatment differed between those who did and did not experience further relapse. Predicting and preventing relapse is one of the main goals of managing patients with MG. Although various attempts have been made to predict the risk factors for relapse,[44,45] it is still difficult to predict whether a future relapse will occur. In the present study, the FABP3 concentration consistently increased in patients who experienced additional relapses. This suggests that monitoring FABP3 concentration after treatment could be used to predict further MG relapses.

Thymectomy is considered for patients with MG to improve control of the disease or for treating a coexisting thymic tumor.[46] In the present study, the concentrations of various myokines in plasma were significantly lower in patients with MG who had undergone thymectomy. The association between low myokine concentration and thymectomy can be explained in two ways. First, certain myokines are known to be produced in the thymus, and surgically removing the thymus may induce a decrease in myokine concentrations. LIF and oncostatin have been found to be expressed in the thymi of patients with MG, and the expression level was correlated with age.[47] FGF21 and fractalkine expression have been observed in the thymi of mouse models.[48,49] These myokines are known to modulate age-related thymic involution or the development of immune cells in the thymus, and the production of these myokines in the thymus could be interrupted after thymectomy. Second, thymectomy may reduce inflammation and therefore decrease the concentration of inflammatory myokines. Previous studies demonstrated the expression of IFN in the thymi of patients with MG and proposed that chronic IFN expression in the thymus promotes inflammatory changes and leads to MG development.[50-52] Myostatin is a proinflammatory myokine that has been found to be associated with the severity of autoimmune diseases such as rheumatoid arthritis,[53] and a high myostatin concentration in patients with MG who have not received thymectomy could be the result of chronic inflammation in the thymus. Although the exact mechanism of the association between thymectomy and myokine concentration cannot be determined based on the present analysis, it should be emphasized that a history of thymectomy should be considered when analyzing myokine concentrations in patients with MG.

The present study had several limitations. First, we analyzed the plasma concentration of 15 myokines, and thus the possibility of false positives should be considered. As a pilot study, the objective of the present study was to determine the gross profile of myokine concentrations in patients with MG. Further studies are required to precisely elucidate the pathomechanism underlying the changes in the myokine profile. Second, although changes in certain myokine concentrations were observed in the plasma, the specific cell group that contributed to these changes remains unclear. Since myokines are also produced in organs other than skeletal muscle, whether the changes in myokine concentrations are actually a response to the disease by muscle fibers remains unclear. However, FABP3 is mostly expressed in skeletal muscle, and FABP3 expression in damaged skeletal muscle has been consistently demonstrated in previous studies.[18,40] Third, various factors can influence plasma myokine concentrations, including exercise, the presence of metabolic disease, renal function, and bone density.[11,54,55] However, most of these factors were not as-

sessed in the present analysis. Fourth, caution is required when interpreting the results for myokines with high CVs or whose concentrations were detected in only a small proportion of the samples.

In conclusion, plasma myokine profiles differed between patients with MG and healthy controls. The myokine concentration was associated with MG duration or a history of thymectomy, and these factors should be considered when analyzing myokine concentrations in patients with MG. Plasma FABP3 concentrations were significantly correlated with MG severity, which suggest an underlying mechanism of muscle damage in patients with MG. Plasma myokines may therefore be useful in understanding MG pathophysiology and assessing MG severity.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

ORCID iDs

Jo Woon Seok	https://orcid.org/0000-0001-9318-3739
Jinny Lee	https://orcid.org/0000-0003-0498-5110
MinGi Kim	https://orcid.org/0000-0002-6921-3085
Min Ju Kim	https://orcid.org/0000-0001-8337-8444
Ha Young Shin	https://orcid.org/0000-0002-4408-8265
Seung Woo Kim	https://orcid.org/0000-0002-5621-0811

Author Contributions

Conceptualization: Jo Woon Seok, Seung Woo Kim. Data curation: Jo Woon Seok, Jinny Lee, MinGi Kim, Min Ju Kim, Seung Woo Kim. Formal analysis: Jo Woon Seok, Jinny Lee, Seung Woo Kim. Funding acquisition: Seung Woo Kim. Investigation: all authors. Methodology: all authors. Project administration: Ha Young Shin, Seung Woo Kim. Resources: Ha Young Shin, Seung Woo Kim. Supervision: Ha Young Shin, Seung Woo Kim. Validation: all authors. Visualization: Jo Woon Seok, Jinny Lee, MinGi Kim, Seung Woo Kim. Writing—original draft: Jo Woon Seok, Jinny Lee, Seung Woo Kim. Writing—review & editing: all authors.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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REFERENCES

1. Feferman T, Maiti PK, Berrih-Aknin S, Bismuth J, Bidault J, Fuchs S, et al. Overexpression of IFN-induced protein 10 and its receptor CXCR3 in myasthenia gravis. *J Immunol* 2005;174:5324-5331.
2. Garcia YR, May JJ, Green AM, Krolick KA. Acetylcholine receptor-reactive antibody induces nitric oxide production by a rat skeletal muscle cell line: influence of cytokine environment. *J Neuroimmunol* 2001;120:103-111.
3. Li H, Shi FD, Bai X, Huang Y, Diab A, He B, et al. Cytokine and chemokine mRNA expressing cells in muscle tissues of experimental autoimmune myasthenia gravis. *J Neurol Sci* 1998;161:40-46.
4. Maurer M, Bougoin S, Feferman T, Frenkian M, Bismuth J, Mouly V, et al. IL-6 and Akt are involved in muscular pathogenesis in myasthenia gravis. *Acta Neuropathol Commun* 2015;3:1.
5. Zhu D, Xiong WC, Mei L. Lipid rafts serve as a signaling platform for nicotinic acetylcholine receptor clustering. *J Neurosci* 2006;26:4841-4851.
6. Iwasa K, Furukawa Y, Yoshikawa H, Yamada M. Caveolin-3 is aberrantly expressed in skeletal muscle cells in myasthenia gravis. *J Neuroimmunol* 2016;301:30-34.
7. Iwasa K, Nambu Y, Motozaki Y, Furukawa Y, Yoshikawa H, Yamada M. Increased skeletal muscle expression of the endoplasmic reticulum chaperone GRP78 in patients with myasthenia gravis. *J Neuroimmunol* 2014;273:72-76.
8. 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;134:869-888.
9. Lee JH, Jun HS. Role of myokines in regulating skeletal muscle mass and function. *Front Physiol* 2019;10:42.
10. Covington JD, Tam CS, Bajpeyi S, Galgani JE, Noland RC, Smith SR, et al. Myokine expression in muscle and myotubes in response to exercise stimulation. *Med Sci Sports Exerc* 2016;48:384-390.
11. So B, Kim HJ, Kim J, Song W. Exercise-induced myokines in health and metabolic diseases. *Integr Med Res* 2014;3:172-179.
12. Hutchinson KA, Mohammad S, Garneau L, McInnis K, Aguer C, Adamo KB. Examination of the myokine response in pregnant and non-pregnant women following an acute bout of moderate-intensity walking. *Front Physiol* 2019;10:1188.
13. Aoi W, Naito Y, Takagi T, Tanimura Y, Takanami Y, Kawai Y, et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut* 2013;62:882-889.
14. Fontes-Oliveira CC, Busquets S, Fuster G, Ametller E, Figueras M, Olivan M, et al. A differential pattern of gene expression in skeletal muscle of tumor-bearing rats reveals dysregulation of excitation–contraction coupling together with additional muscle alterations. *Muscle Nerve* 2014;49:233-248.
15. Vernerová L, Horváthová V, Kropáčková T, Vokurková M, Klein M, Tomčík M, et al. Alterations in activin A-myostatin-follistatin system associate with disease activity in inflammatory myopathies. *Rheumatology (Oxford)* 2020;59:2491-2501.
16. Jørgensen LH, Petersson SJ, Sellathurai J, Andersen DC, Thayssen S, Sant DJ, et al. Secreted protein acidic and rich in cysteine (SPARC) in human skeletal muscle. *J Histochem Cytochem* 2009;57:29-39.
17. Mageriu V, Manole E, Bastian AE, Staniceanu F. Role of myokines in myositis pathogenesis and their potential to be new therapeutic targets in idiopathic inflammatory myopathies. *J Immunol Res* 2020;2020:9079083.
18. Zhang L, Zhou H, Peng Q, Jiang W, Qiao W, Wang G. Fatty acid binding protein 3 is associated with skeletal muscle strength in polymyositis and dermatomyositis. *Int J Rheum Dis* 2017;20:252-260.
19. Jaretzki A 3rd, 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.
20. 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;841:769-772.
21. Wolfe GI, Herbelin L, Nations SP, Foster B, Bryan WW, Barohn RJ. Myasthenia gravis activities of daily living profile. *Neurology* 1999;52:1487-1489.
22. Burns TM, Conaway M, Sanders DB; MG Composite and MG-QOL15 Study Group. The MG Composite: a valid and reliable outcome measure for myasthenia gravis. *Neurology* 2010;74:1434-1440.
23. Klosowska K, Volin MV, Huynh N, Chong KK, Halloran MM, Woods JM. Fractalkine functions as a chemoattractant for osteoarthritis sy-

novial fibroblasts and stimulates phosphorylation of mitogen-activated protein kinases and Akt. *Clin Exp Immunol* 2009;156:312-319.
24. Jones B, Koch AE, Ahmed S. Pathological role of fractalkine/CX3CL1 in rheumatic diseases: a unique chemokine with multiple functions. *Front Immunol* 2011;2:82.
25. Suzuki F, Kubota T, Miyazaki Y, Ishikawa K, Ebisawa M, Hirohata S, et al. Serum level of soluble CX3CL1/fractalkine is elevated in patients with polymyositis and dermatomyositis, which is correlated with disease activity. *Arthritis Res Ther* 2012;14:R48.
26. Matsunawa M, Isozaki T, Odai T, Yajima N, Takeuchi HT, Negishi M, et al. Increased serum levels of soluble fractalkine (CX3CL1) correlate with disease activity in rheumatoid vasculitis. *Arthritis Rheum* 2006;54:3408-3416.
27. Lee JH, Kwok SK, Jung SM, Lee J, Lee JS, Baek SY, et al. Role of fractalkine in the pathogenesis of primary Sjögren syndrome: increased serum levels of fractalkine, its expression in labial salivary glands, and the association with clinical manifestations. *J Rheumatol* 2014;41:2425-2438.
28. Fahy OL, Coates NJ, McColl SR. Inhibition of cytokine-induced fractalkine production by bacterial invasion of human-dermal fibroblasts. *Lab Invest* 2003;83:721-730.
29. Fraticelli P, Sironi M, Bianchi G, D'Ambrosio D, Albanesi C, Stoppacciaro A, et al. Fractalkine (CX3CL1) as an amplification circuit of polarized Th1 responses. *J Clin Invest* 2001;107:1173-1181.
30. Uzawa A, Kawaguchi N, Himuro K, Kanai T, Kuwabara S. Serum cytokine and chemokine profiles in patients with myasthenia gravis. *Clin Exp Immunol* 2014;176:232-237.
31. Yilmaz V, Oflazer P, Aysal F, Durmus H, Poulas K, Yentur SP, et al. Differential cytokine changes in patients with myasthenia gravis with antibodies against AChR and MuSK. *PLoS One* 2015;10:e0123546.
32. Uzawa A, Kuwabara S, Suzuki S, Imai T, Murai H, Ozawa Y, et al. Roles of cytokines and T cells in the pathogenesis of myasthenia gravis. *Clin Exp Immunol* 2021;203:366-374.
33. Strömberg A, Olsson K, Dijksterhuis JP, Rullman E, Schulte G, Gustafsson T. CX3CL1--a macrophage chemoattractant induced by a single bout of exercise in human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 2016;310:R297-R304.
34. Heuckeroth RO, Birkenmeier EH, Levin MS, Gordon JI. Analysis of the tissue-specific expression, developmental regulation, and linkage relationships of a rodent gene encoding heart fatty acid binding protein. *J Biol Chem* 1987;262:9709-9717.
35. Kusudo T, Kontani Y, Kataoka N, Ando F, Shimokata H, Yamashita H. Fatty acid-binding protein 3 stimulates glucose uptake by facilitating AS160 phosphorylation in mouse muscle cells. *Genes Cells* 2011;16:681-691.
36. Lee SM, Lee SH, Jung Y, Lee Y, Yoon JH, Choi JY, et al. FABP3-mediated membrane lipid saturation alters fluidity and induces ER stress in skeletal muscle with aging. *Nat Commun* 2020;11:5661.
37. Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nat Rev Drug Discov* 2008;7:489-503.
38. Sorichter S, Mair J, Koller A, Pelsers MM, Puschendorf B, Glatz JF. Early assessment of exercise induced skeletal muscle injury using plasma fatty acid binding protein. *Br J Sports Med* 1998;32:121-124.
39. McDermott MM, Ferrucci L, Gonzalez-Freire M, Kosmac K, Leeuwenburgh C, Peterson CA, et al. Skeletal muscle pathology in peripheral artery disease: a brief review. *Arterioscler Thromb Vasc Biol* 2020;40:2577-2585.
40. Syed MH, Zamzam A, Khan H, Singh K, Forbes TL, Rotstein O, et al. Fatty acid binding protein 3 is associated with peripheral arterial disease. *JVS Vasc Sci* 2020;1:168-175.
41. Gupta L, Majumder S, Aggarwal A, Misra R, Lawrence A. Serum fatty acid-binding protein 3 levels differentiate active from inactive myositis and correlate with response to therapy. *Indian J Rheumatol* 2020;15:187-190.
42. Plomp JJ, Huijbers MGM, Verschuuren JJGM, Borodovsky A. A bioassay for neuromuscular junction-restricted complement activation by myasthenia gravis acetylcholine receptor antibodies. *J Neurosci Methods* 2022;373:109551.
43. Luo YB, Mastaglia FL. Dermatomyositis, polymyositis and immune-mediated necrotising myopathies. *Biochim Biophys Acta* 2015;1852:622-632.
44. Su S, Lei L, Fan Z, Zhang S, Wen Q, Wang J, et al. Clinical predictors of relapse in a cohort of steroid-treated patients with well-controlled myasthenia gravis. *Front Neurol* 2022;13:816243.
45. Kim KH, Kim SW, Cho J, Chung HY, Shin HY. Anti-titin antibody is associated with more frequent hospitalization to manage thymoma-associated myasthenia gravis. *Front Neurol* 2022;13:978997.
46. 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;96:114-122.
47. Sempowski GD, Hale LP, Sundy JS, Massey JM, Koup RA, Douek DC, et al. Leukemia inhibitory factor, oncostatin M, IL-6, and stem cell factor mRNA expression in human thymus increases with age and is associated with thymic atrophy. *J Immunol* 2000;164:2180-2187.
48. Youm YH, Horvath TL, Mangelsdorf DJ, Kliewer SA, Dixit VD. Prolongevity hormone FGF21 protects against immune senescence by delaying age-related thymic involution. *Proc Natl Acad Sci U S A* 2016;113:1026-1031.
49. Drennan MB, Govindarajan S, De Wilde K, Schlenner SM, Ware C, Nedospasov S, et al. The thymic microenvironment differentially regulates development and trafficking of invariant NKT cell sublineages. *J Immunol* 2014;193:5960-5972.
50. Cufi P, Dragin N, Weiss JM, Martinez-Martinez P, De Baets MH, Roussin R, et al. Implication of double-stranded RNA signaling in the etiology of autoimmune myasthenia gravis. *Ann Neurol* 2013;73:281-293.
51. Payet CA, You A, Fayet OM, Dragin N, Berrih-Aknin S, Le Panse R. Myasthenia gravis: an acquired interferonopathy? *Cells* 2022;11:1218.
52. Lefeuvre CM, Payet CA, Fayet OM, Maillard S, Truffault F, Bondet V, et al. Risk factors associated with myasthenia gravis in thymoma patients: the potential role of thymic germinal centers. *J Autoimmun* 2020;106:102337.
53. Murillo-Saich JD, Vazquez-Villegas ML, Ramirez-Villafaña M, Saldaña-Cruz AM, Aceves-Aceves JA, Gonzalez-Lopez L, et al. Association of myostatin, a cytokine released by muscle, with inflammation in rheumatoid arthritis: a cross-sectional study. *Medicine (Baltimore)* 2021;100:e24186.
54. Ebert T, Focke D, Petroff D, Wurst U, Richter J, Bachmann A, et al. Serum levels of the myokine irisin in relation to metabolic and renal function. *Eur J Endocrinol* 2014;170:501-506.
55. Singhal V, Lawson EA, Ackerman KE, Fazeli PK, Clarke H, Lee H, et al. Irisin levels are lower in young amenorrheic athletes compared with eumenorrheic athletes and non-athletes and are associated with bone density and strength estimates. *PLoS One* 2014;9:e100218.