Figure S2 Growth factors and FBS attenuate the damage to human myogenic cells by alendronate. Hu5/KD3 cells (5×10^4 cells) were cultured in 20% FBS-hDMEM for 2 day and then medium was switched to pmGM, 20% FBS-hDMEM, and pmDM with or without alendronate (ALN, 100 μM) for 5 day further. Scale bar, 100 μm.

Movie S1 Alendronate prevents cell migration and proliferation, resulting in cell detachment. Hu5/KD3 cells were cultured in pmGM. Changes in cell morphology were recorded by phase-contrast time-lapse microscopy in the absence (Movie 1) or presence (Movie 2) of ALN (100 μM). Images were obtained every 10 min during 74–92 h of culture.

Movie S2 Changes in cell morphology were recorded by phase-contrast time-lapse microscopy in the presence of ALN (100 μM). Images were obtained every 10 min during 74–92 h of culture in pmGM with ALN.

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ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Association of decreased sympathetic nervous activity with mortality of older adults in long-term care

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Aim: To investigate the relationship between physical function, mortality and autonomic nervous activity measured by heart rate variability of elderly in long-term care.

Methods: Cross-sectional and longitudinal studies were carried out at hospitals and health service facilities for the elderly in Nagano prefecture, Japan, from July 2007 to March 2011. A total of 105 long-term care older adults and 17 control older adults with independent physical function were included. The Functional Independence Measure (FIM) and Barthel Index were determined as indices of physical function. Twenty-four-hour Holter monitoring was carried out. From RR intervals in electrocardiograms, heart rate and standard deviations of all NN intervals in all 5-min segments of the entire recording, power spectral density, low frequency, high frequency and low frequency/high frequency (LF/HF) were calculated.

Results: FIM score and Barthel Index were 46 ± 26 and 30 ± 31, respectively, in long-term care elderly. FIM and Barthel index were significantly correlated with heart rate and the standard deviations of all NN intervals after adjustment for age, sex, cardiovascular risk factors and FIM. Furthermore, LF/HF was significantly decreased in long-term care elderly compared with control elderly after adjustment for covariates. In addition, decrease in LF/HF was an independent risk factor for mortality.

Conclusion: Low LF/HF activity was observed in long-term care elderly and was related to an increase of overall mortality. **Geriatr Gerontol Int 2014; 14: 159–166.**

Keywords: heart rate variability, long-term care, mortality, motor activity, sympathetic nervous system.

Introduction

The number of older adults who require long-term care (LTC) has been increasing in Japan, and it was reported that there were 4.67 million older adults in LTC in 2008.[1] One of the characteristics of older adults in long-term care is physical and cognitive dysfunction. Physical dysfunction, including slow gait, low handgrip strength, low physical activity, weight loss and exhaustion, are reported to be associated with increased overall mortality.[2] In Japan, LTC elderly is defined as those who require assistance with walking, moving, and washing their face, body and mouth, representing functional disability and high mortality.[3] Thus, it is important to maintain or increase physical function in LTC elderly.

The underlying causes of physical dysfunction in Japanese LTC elderly include cerebrovascular disease, dementia, fractures, falls, weakness as a result of aging, and arthritis.[3] Recent studies have shown that these diseases with physical dysfunction are associated with low sympathetic nervous system activity.[4–7]

Skin sympathetic reactivity (SSR) reflects sympathetic nervous system activity. Muslumanoglu *et al.* showed that low SSR was associated with greater severity of paralysis, and depression of sympathetic reflex activity was associated with moderate or severely limited motor function in the chronic phase of ischemic cerebrovascular disease in elderly patients.[5] In addition, low plasma norepinephrine and low iodine-131-meta-iodobenzylguanidine (^{123}I-MIBG) uptake were observed in patients with Lewy body dementia compared with normal healthy subjects.[6,7] RR intervals in the electrocardiogram are utilized to evaluate heart rate variability

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(HRV), which reflects autonomic nervous system activity.[8] In practice, low frequency/high frequency (LF/HF), a marker of sympathovagal balance or sympathetic modulation, showed a positive correlation with respiratory and skeletal muscle strength in chronic obstructive pulmonary disease.[4] Furthermore, decreased LF/HF was related to overall mortality in frail older adults.[9]

In addition to measurement of SSR, norepinephrine spillover and ^{123}I-MIBG scintigraphy uptake, HRV has recently been used as a marker of autonomic nervous function.[8] HF was reported to reflect parasympathetic nervous system activity and LF/HF to represent sympathovagal balance or sympathetic modulation. In addition, decreased HRV was associated with cardiovascular disease (CVD),[10] cardiac death[11] and all-cause mortality.[9] Whereas HRV is known to decrease with the aging process,[12,13] little is known about the relationship between sympathetic nervous activity and mortality in LTC elderly.

In the Framingham heart study, a cohort study in American community-dwelling people, mortality and HRV were investigated in older adults, and it was not shown that low LF/HF correlated with mortality,[14] whereas in a cohort study of frail older adults, low LF/HF was significantly correlated with both frailty and mortality in the Women's Health and Aging Study-I (WHAS-I).[9]

Aging attenuates sympathetic nervous modulation,[12,13] and previous studies suggested that low sympathetic nervous activity might be associated with physical and cognitive dysfunction. However, only some of the subjects were frail or LTC elderly,[9,14] and there is little evidence describing the relationship between physical function, mortality and sympathetic nervous activity in LTC elderly. In particular, few studies have focused on the specific characteristics of sympathetic nervous activity in LTC elderly. Therefore, we investigated the relationship between sympathetic nervous activity, measured by HRV, and physical function and mortality in older adults in LTC.

Methods

Study design and participants

The present observational study analyzed 105 consecutive older adults in LTC aged 75 years or older who were admitted to a rehabilitation unit or a health service facility for older adults that provided rehabilitation. All hospitals and health service facilities were located in Nagano prefecture, Japan. Inclusion criteria were older adults in LTC aged 75 years or older receiving rehabilitation. Exclusion criteria were treatment of acute phase diseases within the past 2 weeks, arrhythmia, administration of anti-arrhythmia drugs or β-blockers, malignancy and neurodegenerative diseases other than dementia. As a control for the present study, we recruited 17 elderly outpatients with intact activities of daily living (ADL) who were matched for age, sex and CVD risk factors. The same inclusion and exclusion criteria were used for these control subjects. Medical records were reviewed to obtain information about the medical history of CVD, such as hypertension, diabetes mellitus, hyperlipidemia, chronic heart failure and ischemic heart disease, which was confirmed by the patients or their family. The present study protocol was approved by the institutional review board of the facility. Written informed consent was obtained from all participants or their families.

Heart rate variability

Ambulatory Holter recording was carried out for 24 h using QR2100 (Fukuda ME Kogyo, Tokyo, Japan) and processed with HS1000VL (Fukuda ME Kogyo). For time domain analysis, the standard deviations of all NN intervals in all 5-min segments of the entire recording (SDANN) were calculated, and frequent domain analysis was carried out with fast Fourier transform. From the power spectral density, low frequency (LF; 0.04~0.15 Hz), high frequency (HF; 0.15~0.40 Hz) and low frequency/high frequency (LF/HF) were determined.

Anthropometric, physical function and hematological measures

Height, weight and body mass index (BMI) were measured before Holter monitoring. The Functional Independence Measure (FIM)[15] and Barthel Index[16] were determined in order to assess physical function. Venous blood samples were obtained from participants in the morning after an overnight fast. Blood cell counts and serum levels of chemical parameters were determined by a commercial laboratory (Health Science Research Institute, Yokohama, Japan).

Statistical analysis

Data were analyzed using SPSS software version 11.0.1J (SPSS Japan, Tokyo, Japan). Mann–Whitney *U*-test for continuous variables and χ^2-test for categorical variables were used to compare controls and LTC elderly. Pearson's correlation coefficient was calculated, and standardized multiple regression analysis of HRV indices was carried out with age, sex, FIM, Barthel Index and blood nutritional data as covariates. Multiple regression analysis was used to calculate Cox hazard ratio, with adjustment for age, sex, clinical risk factors and FIM. Kaplan–Meier survival rate was computed for HRV indices.

Table 1 Characteristics of long-term care elderly and healthy elderly controls

	LTC elderly	Controls	*P*
No. participants	105	17	
Age (years)	86.5 ± 6.0 (75–100)	86.3 ± 9.1 (75–103)	0.311
Sex, male (%)	29 (27.6)	6 (35.3)	0.999
Body mass index	19.5 ± 3.3	22.0 ± 3.5	0.009
Cardiovascular risk factors, *n* (%)			
Hypertension	57 (54.3)	11 (64.7)	0.590
Diabetes mellitus	13 (12.4)	2 (11.8)	0.999
Hyperlipidemia	14 (13.3)	3 (17.6)	0.921
Chronic heart failure	12 (11.4)	1 (5.9)	0.792
Ischemic heart disease	15 (14.3)	1 (5.9)	0.572
Physical function			
FIM	46 ± 26	116 ± 24	<0.001
Barthel Index	30 ± 31	92 ± 16	<0.001
Blood nutritional data			
Albumin (g/dL)	3.5 ± 0.5	3.9 ± 0.3	<0.001
Hemoglobin (g/dL)	12.0 ± 1.8	12.4 ± 2.2	0.188
Total cholesterol (mg/dL)	177 ± 40	175 ± 34	0.892
Heart rate variability indices			
SDANN	85.0 ± 34.3	112.1 ± 27.2	0.001
Heart rate (b.p.m.)	73.1 ± 12.1	71.5 ± 7.4	0.878
LF (ms^2)	36.1 ± 25.3	42.4 ± 37.5	0.274
HF (ms^2)	65.9 ± 56.3	60.7 ± 52.3	0.813
LF/HF	0.69 ± 0.27†	0.87 ± 0.31	0.023

Values are mean ± standard deviation. †After adjusted for age, sex, cardiovascular risk factors and Function Independent Measure (FIM), low frequency/high frequency (LF/HF) were significantly lower in long-term care elderly than healthy controls ($P = 0.049$). HF, high frequency; LF, low frequency; SDANN, standard deviations of the all NN intervals in all 5-min segments of the entire recording.

Results

We registered 105 elderly in LTC, and assessed HRV from 24-h Holter monitoring. The underlying diseases of older adults in LTC for rehabilitation were cerebrovascular disease ($n = 59$, 56.2%), disuse syndrome ($n = 26$, 24.8%), fracture ($n = 19$, 18.1%) and dementia ($n = 1$, 1.0%). The proportions of underlying diseases were similar to those reported in Japanese older adults in LTC.[3]

The background data of the present study are shown in Table 1. In LTC elderly, mean age was 86.5 ± 6.0 years, blood nutritional data including albumin, hemoglobin and total cholesterol were at the lower limit of the normal range, and physical function represented by FIM and Barthel Index was significantly lower (46 ± 26 and 30 ± 31, respectively) than that in elderly controls (116 ± 24 and 92 ± 16, respectively). Scores for each FIM item were as follow: eating 3.7 ± 2.2, grooming 2.6 ± 1.8, bathing 1.5 ± 1.1, upper body dressing 2.5 ± 1.7, lower body dressing 2.2 ± 1.6, toileting 2.7 ± 2.0, bladder management 2.6 ± 2.1, bowel management 2.4 ± 2.0, bed to chair transfer 3.0 ± 1.9, toilet transfer 2.4 ± 1.7, shower transfer 1.5 ± 1.4, locomotion (ambulatory or wheelchair level) 2.0 ± 1.8, stairs 1.2 ± 0.8, cognitive comprehension 3.6 ± 2.2, expression 3.6 ± 2.2, social interaction 3.2 ± 2.2, problem solving 2.8 ± 1.9 and memory 2.8 ± 1.9. These score showed that the overall participants required moderate care supporting physical and cognitive function. In addition, BMI, albumin, SDANN and LF/HF were significantly decreased in LTC elderly compared with elderly controls. After adjustment for covariance, of all HRV indices, only LF/HF was significantly lower in LTC elderly (Table 1). Data of HRV indices were obtained every 5 min, and averaged every 3 h to examine the circadian rhythm in both LTC elderly and healthy controls. A significant decrease of LF/HF was observed in the night-time in healthy controls, whereas there was a loss of circadian rhythm in LTC elderly (Fig. 1).

Multiple regression analysis showed that the associations between heart rate, SDANN and physical function (Barthel Index and FIM) were independent of age, sex, and CVD. Furthermore, albumin and hemoglobin were also correlated with heart rate and SDANN. In contrast, LF, HF and LF/HF were not significantly correlated with physical function and blood nutritional data (Table 2).

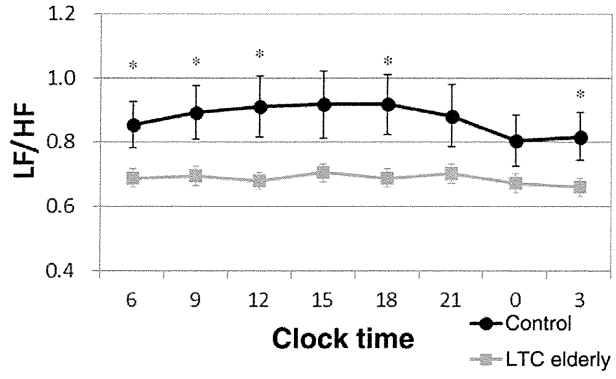

Figure 1 The activity of low frequency/high frequency (LF/HF) in long-term care (LTC) elderly and controls. The RR interval data were measured every 5 min, and averaged every 3 h. *$P < 0.05$, mean ± SEM,

Next, we followed the survival of LTC elderly, and 23 people died among 105 LTC elderly during a mean follow-up period of 8.9 months. The major cause of death was pneumonia ($n = 12$). There was no sign of stroke among the study participants, and one participant with acute myocardial infarction was observed during the follow-up period. Mortality according to HRV indices divided by the average is shown in Table 3. After adjustment for covariates, of all HRV indices, only LF/HF was associated with mortality. Kaplan–Meier survival curves also showed an association between decreased LF/HF and high mortality (Fig. 2). In addition to adjusted covariates, BMI, Barthel Index, and blood nutritional data were not different between the high LF/HF group and low LF/HF group (data not shown).

Discussion

In the present study, we investigated the relationship between physical function, mortality and sympathetic nervous activity measured by HRV in Japanese LTC elderly, and it was shown that LF/HF was significantly decreased in LTC elderly after adjustment for age, sex, CVD risk factors and FIM compared with elderly controls. In addition, the circadian rhythm of LF/HF was lost in LTC elderly, and low LF/HF was associated with overall mortality.

In a previous study, low LF/HF was associated with both frailty and mortality in community-dwelling people of whom one-third were frail elderly,[9] and these associations were consistent with the present data. Additionally, low LF/HF was also shown in LTC elderly, and was independent of physical function.

Elevated heart rate or low SDANN leads to cardiovascular disease and low physical function,[17,18] and the same relationship was also observed in LTC elderly. Furthermore, low albumin and low hemoglobin were observed in the high heart rate group, and limited physical function was observed in LTC elderly. These results are supported by a previous report.[19] So it might be possible to improve the physical function of LTC elderly by maintaining their nutritional state. The high LF/HF group has been reported to show high physical function and muscle mass,[4,20] whereas the present data did not show this association. One of the reasons for this discrepancy is thought to be the effect of aging. Aging generally attenuates LF/HF, and the patients in the present study were older than those in other studies.[9,14] Another reason might be autonomic nervous system disturbance. In particular, the circadian rhythm of LF/HF was impaired in LTC elderly.

Circadian imbalance of LF/HF has been shown in some disorders, such as Parkinson's disease, type 2 diabetes mellitus (T2DM) and ischemic stroke;[21–23] and furthermore, physical activity also influences HRV indices.[24,25] In the present study, LTC elderly with Parkinson's disease were excluded, and CVD risk factors including T2DM were matched between LTC elderly and healthy controls, as stroke and physical activity might affect LF/HF. However, the influence of both conditions on LF/HF is controversial. High physical activity and good posture led to high LF/HF activity,[26] whereas it was also suggested that LF/HF was not affected by physical activity.[13] The effect of LF/HF on stroke is also controversial.[23,27,28] In ischemic stroke patients, LF/HF was higher than healthy controls in some studies,[27,28] whereas another study suggested that LF/HF was lower in patients.[23] So the mechanism of LF/HF circadian rhythm disturbance is not clear, though its recovery might be important to increase physical function in LTC elderly. Other reasons why LF/HF and physical function did not show a correlation in LTC elderly might to be the effects of stroke, insufficient exposure to daylight and posture at daytime. All participants were aged over 75 years in the present study, and there is a possibility that asymptomatic lacunar infarction might be observed. It has also been suggested that lacunar infarction disturbs the autonomic nervous system, leading to a decrease in LF/HF and the related value of the autonomic nervous system, resulting in a disappearance of the correlation between physical activity and LF/HF. In addition, exposure to daylight was known to be one of the most powerful rhythmic regulators in the environment.[29] All participants in the present study spent their time indoors for rehabilitation and care. Furthermore, it is known that the supine position increases HF and decreases LF/HF,[30] and LTC elderly participants who were at rehabilitation units or health service facilities might spend more time in bed compared with outpatient controls, leading to low LF/HF and disappearance of the correlation between LF/HF and physical activity in the present study.

Table 2 Multiple regression analysis of heart rate variability indices with physical function and blood nutritional data after adjusted for age, sex and cardiovascular risk factors

	HR	SDANN	LF	HF	LF/HF
FIM	−0.25*	0.28*	0.19	0.15	−0.08
Barthel Index	−0.27*	0.29*	0.08	0.04	0.00
Body mass index	−0.05	0.05	0.00	−0.08	0.19
Albumin	−0.21*	0.25*	0.05	−0.02	0.11
Hemoglobin	−0.20*	0.27*	0.12	0.12	0.05
Total cholesterol	−0.01	−0.05	−0.13	−0.17	0.03

*$P < 0.05$, analyzed in 105 long-term care elderly. FIM, function independent measure; HF, high frequency; HR, heart rate; LF, low frequency; SDANN, standard deviations of the all NN intervals in all 5-min segments of the entire recording.

Table 3 Proportional hazards regression analysis of the impact of heart rate variability measure on overall mortality

	Hazard ratio†	95% Confidence interval	*P*
Unadjusted			
SDANN (ms)	1.84	0.77–4.38	0.171
LF (ms^2)	1.61	0.59–4.38	0.353
HF (ms^2)	2.14	0.72–6.34	0.169
LF/HF	4.73	1.59–14.06	0.005
Age, sex and cardiovascular risk factors adjusted for association with mortality			
SDANN (ms)	1.53	0.60–3.86	0.372
LF (ms^2)	1.65	0.57–4.78	0.357
HF (ms^2)	2.60	0.82–8.22	0.105
LF/HF	3.37	1.02–11.07	0.046
Age, sex, FIM and cardiovascular risk factors adjusted for association with mortality			
SDANN (ms)	1.19	0.44–3.17	0.736
LF (ms^2)	1.49	0.50–4.41	0.475
HF (ms^2)	2.85	0.83–9.83	0.097
LF/HF	3.61	1.08–12.10	0.038

Based on 23 deaths among 105 participants. Mean values of heart rate variability measure are in Table 1. †Hazard ratio of death rates of participants whose heart rate variability were less than average. FIM, function independent measure; HF, high frequency; HR, heart rate; LF, low frequency; SDANN, standard deviations of the all NN intervals in all 5-min segments of the entire recording.

Recent studies showed that decreased HRV indices including LF, HF and LF/HF were associated with CVD risk factors, and decreased LF was an independent predictor of death in elderly people.[31,32] However, the present findings showed that, of all HRV indices, only LF/HF was associated with mortality. This result is supported by a previous study in which, of HRV indices, LF/HF was associated with both frailty and mortality.[9] The major difference between the present study and other studies is whether or not the participants included frail LTC elderly. All participants were LTC elderly in the present study and WHAS-I, which was reported by Varadhan *et al.* and consisted of one-third frail elderly, whereas in other studies the participants were community-dwelling older adults with intact ADL, and they did not consider physical function.[14,32,33] These results suggest that the significance of LF/HF might differ between LTC elderly and elderly with intact ADL and physical function.

There is a discrepancy in the results derived from studies of LTC elderly and studies of elderly with intact physical function regarding sympathetic nervous activity. Exercise activates the sympathetic nervous system, leading to an increase in blood pressure, muscle blood

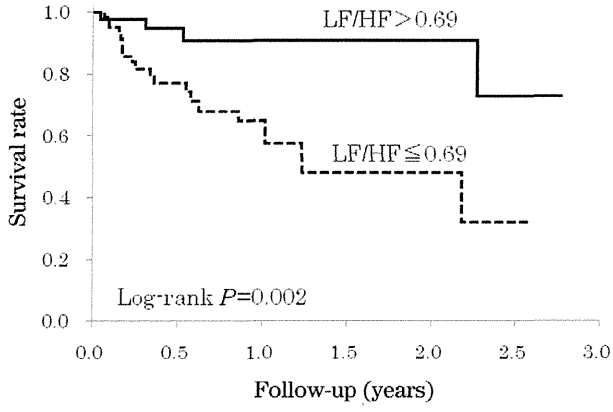

Figure 2 Kaplan–Meyer survival curves for death according to low frequency/high frequency (LF/HF). Mortality was significantly higher for patients with low LF/HF than for patients with high LF/HF. The mean follow-up period was 8.9 months.

flow and muscle strength by inducing muscle protein synthesis,[34–37] suggesting that low sympathetic nervous activity is related to not only physical dysfunction, but also the inability to maintain muscle strength, leading to a worse outcome in LTC elderly. Appropriate activation of the sympathetic nervous system might prevent muscle wasting and improve overall mortality in LTC elderly.

Activation of the sympathetic nervous system has been applied to aging or sarcopenic model rats. The β2-adrenergic agonists, clenbuterol and formoterol, improved muscle mass and muscle strength, and prevented muscle aging in aging, disuse and sarcopenia[38–44] model rats. In contrast, inhibition of sympathetic nervous activity with β-blockers was associated with a worse outcome in older adults.[45] These findings also suggest the importance of preventing a sympathetic nervous activity decline in LTC elderly.

There were several study limitations. First, this was an observational study, and could not provide direct evidence of causality. So it will be necessary to carry out randomized controlled trials to show whether high sympathetic nervous activity leads to a good outcome or not. Second, excessive sympathetic nervous activity is associated with cardiovascular risk factors, such as hypertension, left ventricular myocardial hypertrophy and old cerebrovascular disease.[46,47] In addition, the number of control subjects was relatively small in the present study. Based on these results, it might be hard to apply the findings in the present study to the oldest old population in general. However, some studies, particularly in the elderly, showed that decreased sympathetic nervous activity was associated with a worse outcome.[9] In addition to low physical activity, poor handgrip strength and frailty are known to be important risk factors predicting death older adults,[2,48–50] and few reports have focused on LTC elderly. Therefore, the present study has the possibility of providing evidence to improve physical function and mortality in LTC elderly by means of maintaining or increasing LF/HF.

In summary, the present study showed that LF/HF is a factor that distinguishes LTC elderly from elderly controls independent of physical function. In addition, the circadian rhythm of LF/HF was lost in LTC elderly. Furthermore, low LF/HF was associated with high mortality. For LTC elderly aged 75 years or over, LF/HF might be a predictive biomarker of physical function and mortality.

Disclosure statement

There is no financial support or relationship that might pose conflicts of interest.

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Review

Mechanisms associated with the pathogenicity of antibodies against muscle-specific kinase in myasthenia gravis

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ABSTRACT

The presence of autoantibodies against muscle-specific kinase (MuSK) at the neuromuscular junction (NMJ) results in myasthenia gravis (MG). MuSK antibody-associated MG (MuSK MG) patients often have severe symptoms, including bulbar dysfunction, respiratory insufficiency and atrophy of the facial and tongue muscles. MuSK antibodies in MG patients predominantly belong to the IgG4 subclass, and the unique properties of IgG4 antibodies are directly associated with the pathogenic mechanisms of MuSK MG. Histopathological studies in animal models of MuSK MG have revealed that anti-MuSK antibodies cause contraction of motor terminals, significant loss of acetylcholine receptor (AChR) expression, and a reduction in synaptic folds at the postsynaptic membrane in the absence of complement involvement. Failure of neuromuscular transmission at pre- and post-synaptic membranes of the NMJs has been observed in both patients and animal models of MuSK MG. A murine model of MuSK-MG revealed the mechanisms underlying cholinergic hypersensitivity after administration of acetylcholinesterase inhibitors, which has also been observed in MuSK-MG patients. Further studies of this model have provided evidence suggesting that 3,4-diaminopyridine may be effective as a symptomatic therapy for MuSK MG.

Contents

1. Introduction

Myasthenia gravis (MG) is caused by autoantibodies that target the postsynaptic membranes of neuromuscular junctions (NMJs), cholinergic synapses that connect nerve terminals and skeletal muscle fibers [1–5]. In 2001, antibodies against muscle-specific kinase (MuSK) were identified in patients with generalized, acetylcholine receptor (AChR) antibody-negative MG [6,7]. MuSK, a receptor tyrosine kinase, is expressed at NMJs from the earliest stages of synaptogenesis and is required for the formation of NMJs during development [8–10]. Together with low-density lipoprotein receptor-related protein 4 (LRP4), MuSK functions as a receptor for agrin, a motor-neuron-derived matrix proteoglycan. MuSK is activated by dimerization with LRP4 upon agrin binding [11–13]. However, the mechanisms associated with NMJ formation and maintenance that are regulated by these molecules are not fully understood [14].

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2. Pathogenicity of MuSK antibodies

The clinical features of patients with MuSK antibody-positive MG (MuSK MG) are distinctive. These patients often have severe bulbar dysfunction and respiratory insufficiency that can be difficult to treat effectively with immunosuppressive and immunomodulatory strategies. Atrophy of the facial and tongue muscles is also common [15–24]. However it has been disputed whether MuSK antibodies were pathogenic agents or just by standing disease makers. This controversy was primarily due to the lack of a model of experimental autoimmune myasthenia gravis (EAMG) for MuSK-MG [25,26].In 2006, the first piece of evidence showing that MuSK antibodies could induce MG was demonstrated by active immunization of rabbits with purified MuSK extracellular domain [27]. MG was also induced in mice and rats by active immunization with MuSK proteins [28–30] and in mice by intra-peritoneal injection of a high dose of human total IgG purified from the plasma of MuSK-MG patients [31]. Accumulated evidence from these experimental animal models of MuSK MG has since confirmed the pathogenicity of anti-MuSK antibodies [32–34].

3. Pathohistological features of MuSK MG

AChR loss, immune-complex deposition (IgG and complement), and the destruction of postsynaptic membranes at NMJs are typical pathohistological findings in MG patients with anti-AChR antibodies [35–37]. In contrast to AChR antibody-positive MG (AChR MG), morphological studies of NMJs in patients with MuSK MG have not clearly delineated the pathohistological features associated with the disorder. Previous studies have reported an absence of histological abnormalities in MuSK-MG patients, despite analysis of AChR density and pathological changes in NMJ structures [26,38], whereas MuSK antibodies from patients are experimentally capable of reducing AChR clustering in myotubes [6].

Morphological defects observed in the NMJs of MuSK-MG animal models are noteworthy in comparison with results from histological studies of MuSK-MG patients (Fig. 1). Significant loss of AChR expression, retraction of motor terminals apposing AChR clusters, and reduction of synaptic folds at postsynaptic membranes have been observed in animal models positively immunized with MuSK proteins or passively transferred with a purified IgG fraction from plasma of MuSK-MG patients [27,28,30,39,40]. The clinical signs present in these animal models, including muscle weakness and atrophy, were extremely severe, demonstrating the pathogenicity of MuSK antibodies. In contrast, histological studies of MuSK-MG patients were performed using muscle biopsies from costal muscles during periods of relatively stable clinical symptoms or from limb muscles, which show less marked clinical changes in MuSK-MG patients [26,38]. Therefore, quantitative histological analyses of NMJs isolated from more strongly affected muscles of patients are needed for comparative studies between patients and animal models of MuSK MG.

4. Failures in neuromuscular transmission at NMJs in MG

Electromyography (EMG) studies in animal models of MuSK MG have provided evidence of a defect in neuromuscular transmission at NMJs [41]. Repetitive stimulation of a mixed nerve at 3 Hz using needle electrodes evokes a compound muscle action potential (CMAP), which can be recorded by needle electrodes in muscles. The amplitude and area of the CMAP are a function of the number of muscle fibers activated by each stimulus [41]. Decrement in CMAP amplitude has been observed in muscles of animal models of MuSK MG during repetitive nerve stimulation and has also been observed in mild-to-severely affected muscles of MuSK-MG patients [22,27,28,31,39,40]. CMAP decrement in EMG is indicative of the progressive failure of successfully transmitting NMJs during repetitive stimulation and is thought to represent the observed fatigable muscle weakness in animal models.

Intracellular recordings from muscle fibers of excised hemidiaphragms of animal models or biopsies from patient muscles can assess the precise transmission defects present at NMJs [42,43]. *Ex vivo* electrophysiological studies have indicated that failures in both pre- and postsynaptic transmission cause fatigable muscle weakness in animal models and patients with MuSK-MG [39,40,44–46]. This observation indicates that antibodies against MuSK disturb both pre- and postsynaptic functions, despite the observation that MuSK is expressed only at postsynaptic NMJ membranes, along with AChR and LRP4. MuSK and LRP4 have both been demonstrated to be required for presynaptic, as well as postsynaptic, NMJ formation during development [10,11]. Recent studies have demonstrated that LRP4 acts as a retrograde signaling molecule to modulate presynaptic functions and structures [47,48]. Therefore, further studies are needed to fully elucidate the mechanisms associated with failure of neuromuscular transmission at NMJs caused by anti-MuSK antibodies.

5. The IgG4 subclass of MuSK-MG antibodies

Anti-MuSK antibodies in MG patients predominantly belong to the IgG4 subclass, which has distinct features compared with other human IgG subclasses [49]. The unique properties associated with IgG4 function and structure are directly associated with the pathogenic mechanisms of MuSK MG.

5.1. Pathogenicity in the absence of complement activation

Although MG severity is correlated with the titer of anti-MuSK IgG4 [50], the human IgG4 subclass of antibodies does not activate the classical complement pathway [19,51,52]. This situation differs from that of AChR MG, in which complement-mediated damage to the postsynaptic membrane is considered to be a major source of pathogenicity [53,54]. Recent studies in complement-deficient mouse strains actively immunized with MuSK protein have demonstrated that involvement of the complement system is not required for the onset of MuSK-MG [39]. Immmunized, complement C3- and C5-deficient mouse strains both developed severe muscle weakness, loss of AChR expression, reductions in the size of motor terminals opposing AChR clusters at NMJs, and decrement EMG patterns, similar to phenotypes typically observed in MuSK-MG patients (Table 1). In contrast, mice that were passively transferred with purified IgG1-3 fractions from the plasma of MuSK-MG patients did not develop muscle weakness, whereas those that were transferred with a purified IgG4 fraction developed MuSK MG [40]. Together, these results indicate that anti-MuSK IgG4 antibodies cause MG in the absence of complement activation [32–34].

5.2. Divalent and monovalent anti-MuSK antibodies

The human IgG4 subclass is comprised of a group of dynamic antibodies that exchange Fab arms with other antibodies within this subclass in circulation, resulting in a functionally-monovalent antibody carrying two different antigen-binding sites [55]. The exchange reactions require several days to reach equilibrium, and the kinetics of the exchange are dependent upon endogenous IgG4 levels [56]. In the case of MuSK MG, the result of half-antibody exchange is that patients may have two types of anti-MuSK IgG4 antibodies that contain two different Fab arms, leading to the production of both monovalent and divalent antigen-binding sites for MuSK antigens. Divalent anti-MuSK IgG antibodies can crosslink and activate tyrosine phosphorylation of MuSK in the absence of agrin [27,31,39,57]. In addition, *in vitro* experimental data have suggested that these antibodies may also interfere with MuSK function by inducing internalization of MuSK from the cell surface and/orby accelerating down regulation of the cytoplasmic adaptor protein Dok-7 [57,58], which is indispensable for MuSK function [59]. In contrast, monovalent anti-MuSK antibodies inhibit autophosphorylation of MuSK induced by agrin [39,58] (Table 2).

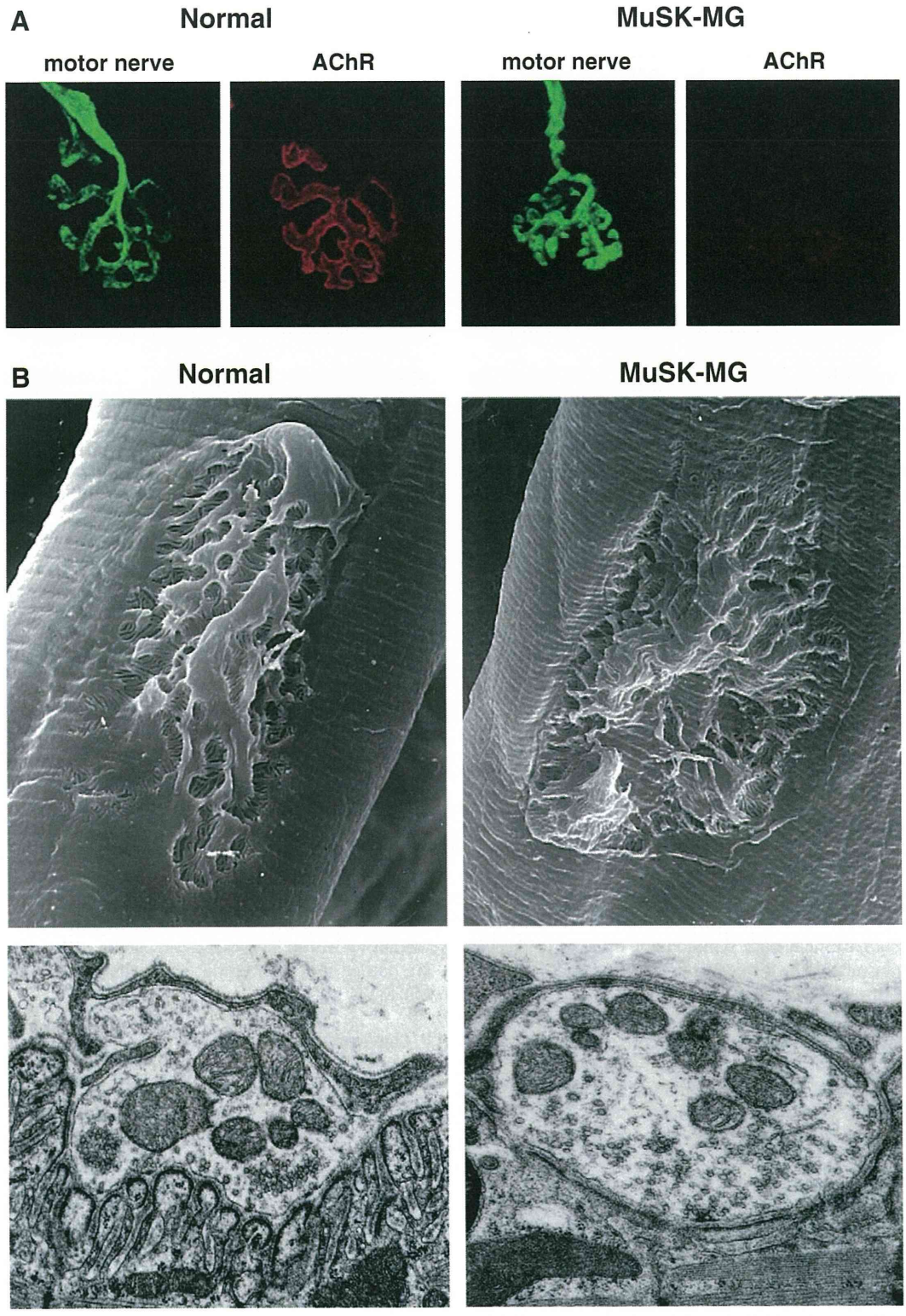

Fig. 1. Morphological defects in NMJs from MuSK-MG mice. (A) Motor nerves (green) were stained with antibodies directed against neurofilaments and synaptophysin. AChR (red) was labeled with rhodamine-α-bungarotoxin. Dim AChR clusters were dispersed in the NMJs of MuSK-MG mice, and motor terminals were retracted compared to those observed in normal control mice. (B) NMJ ultrastructure was observed by scanning (SEM, upper images) or transmission (TEM, bottom images) electron microscopy. SEM images revealed that deep synaptic gutters containing slit-like synaptic folds were less prevalent in NMJs of MuSK-MG mice. In addition, marked loss of synaptic folds beneath motor terminals was observed in MuSK-MG mice *via* TEM.

Monovalent antibodies may also interfere with MuSK dimerization and/or interactions between MuSK and LRP4-agrin [60]. Therefore, these data suggest that monovalent and divalent MuSK IgG4 antibodies may cause MuSK MG through distinct mechanisms.

5.3. Control of IgG4 production in MuSK MG

Production of the human IgG4antibody subclass is promoted by Th2 cytokines (IL-4 and IL-10) [56]. The IgG4 subclass may counteract

Table 1
Incidence of MuSK EAMG in several mouse strains.

Strains	Total no. of mice	MG symptoms (no. of mice)			Incidence (%)	IgG subclasses
			Significant CMAP decrement			
		No symptoms	With <20% weight loss	With >20% weight loss		
A/WySnJ	30	0	0	30	100	1 > 2a, 2b
A/J	5	0	0	5	100	1 > 2b > 2a
A/HeJ	4	0	0	4	100	1 > 2a, 2b
DBA/2	5	0	1	4	100	1 > 2a, 2b
FVB/N	5	0	0	5	100	1 > 2b > 2a
C3-deficient	5	0	5	0	100	1 > 2b > 2a
C57BL/6	11	6	4	1	45.5	1, 2b > 2a
B10.A-*H2*[a]	4	0	4	0	100	1, 2b > 2a
BALB/c	4	1	1	2	75.0	1 > 2a, 2b

the inflammatory properties of other subclasses of antibodies. The regulatory mechanisms associated with MuSK IgG4 production are not well understood. Intriguingly, isotype analysis of a mouse MuSK-MG model revealed that IgG1 was the predominant subclass in all nine mouse strains tested (Table 1). Mouse IgG1 is functionally similar to human IgG4 in terms of electrophoretic mobility and Th2 dependency for production [61], implying that the murine model shares a common immune regulatory mechanism with MuSK-MG patients with respect to the generation of anti-MuSK antibodies [39].

Analysis of a murine model of MuSK MG revealed that complement-deficient mouse strains develop severe MuSK-induced MG with an efficiency of 100%, while complement-sufficient mouse strains are less susceptible [39]. These observations suggest that the complement system modulates self-tolerance immunity and severity of MuSK MG *via* unknown mechanisms [62].

6. Evaluation of novel medications using an animal model of MuSK MG

Clinically, MuSK-MG patients tend to have severely weak and atrophied muscles, dysphagia, and respiratory insufficiency. The severe form of MuSK-MG requires emergent and aggressive therapy to manage respiratory distress [63]. In general, most MG patients undergo first-line symptomatic treatment until immunosuppressive treatment is effective [20,64]. The goal of treatment with symptomatic drugs is to recover neuromuscular transmission by increasing presynaptic transmitter release and potentiating postsynaptic effects.

Acetylcholinesterase (AChE) inhibitors can potentiate postsynaptic effects of acetylcholine (ACh) by preventing its degradation at NMJs, thereby improving symptoms in patients with AChR MG. In contrast, MuSK-MG patients treated with AChE inhibitors often develop cholinergic crises, which are characterized by muscle cramps, fasciculation, and worsening of dysphagia and respiratory distress [20,64–67]. MuSK has been shown to be required for anchoring of AChE at the postsynaptic membrane of NMJs in *in vitro* studies [68]. Consistent with this observation, *in vivo* studies have shown that anti-MuSK antibodies interfere with the binding of AChE at NMJs [69]. A recent study further demonstrated that administration of AChE inhibitors to mice with MuSK MG resulted in hypersensitivity to ACh, similar to that observed in patients with decreased expression of AChE at NMJs [39]. These results suggest that only low-dose AChE inhibitors should be used for treatment of MuSK MG in order to avoid side effects.

In models of MuSK MG, *in vitro* electrophysiological studies have revealed that decreased ACh release from presynaptic membrane occurs at NMJs [39,40,44–46], suggesting a potential role for presynaptic potentiation as a symptomatic therapy for MuSK-MG patients. The agent 3,4-diaminopyridine (3,4-DAP) is the drug of choice for treatment of Lambert–Eaton myasthenic syndrome, an autoimmune disorder affecting the presynaptic side of NMJs [70]. In these patients, autoantibodies against a voltage-gated calcium channel at the presynaptic membrane of NMJs impair stimulated release of ACh from nerve terminals [1,71,72]. The effects of 3,4-DAP have been proposed to result from its function as a potassium channel blocker, which result in an increase in the duration of action potentials, Ca^{2+}influx, and neurotransmitter release [73–75]. Additionally, 3,4-DAP was also reported to stimulate voltage-gated calcium channels and potentiate neurotransmitter release [76]. In a murine model of MuSK MG, treatment with 3,4-DAP was shown to improve both presynaptic neurotransmitter release and postsynaptic effects, suggesting combination therapy with 3,4-DAP and a low dose of AChE inhibitors could serve as a symptomatic therapy for treatment of MuSK-MG patients [77]. Detailed clinical studies are needed to further elucidate the effects of 3,4-DAP in treatment of MuSK MG.

Table 2
Effects of divalent and monovalent MuSK antibodies on agrin-induced activities *in vitro*.

	Agrin alone	Agrin + divalent antibodies	Agrin + monovalent antibodies
MuSK internalization[a]	+	+	n.d.
MuSK phosphorylation	+	+	−
Dok-7 phosphorylation	+	+	−
Dok-7 expression	↓	↓↓	No change
AChRβ phosphorylation	+	+	−
AChR clustering	↑	↓	↓

n.d., not determined.
[a] MuSK internalization results were reported in Cole et al. [57]. All other results were obtained by treating C2C12 myotubes with 1 nM agrin in the presence or absence of monovalent or divalent antibodies targeting MuSK in Mori et al. [58].

7. Conclusions

What are the autoimmune mechanisms underlying MuSK-MG? Why does the IgG4 subclass comprise the predominate group of anti-MuSK antibodies in patients? How do anti-MuSK antibodies interfere with both pre- and post-synaptic functions at NMJs? All of these questions remain to be solved. Further characterization of animal models of MuSK MG is essential for elucidation of the pathogenic mechanisms associated with this disorder, as well as for assessment and development of novel therapeutic strategies for patient treatment. Among currently available models, the complement-deficient mouse model of MuSK MG is particularly useful [39], as 100% of these mice synchronously develop MuSK MG within a month after immunization with MuSK protein. Clinical signs and pathophysiological data observed in MuSK-MG patients have been successfully reproduced in this model. Therefore, as the disease progression is predictable in this animal model, it may be useful for the development and testing of novel therapeutic strategies, in addition to its use in pathophysiological studies.

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Platelet-associated antibodies, cellular immunity and FCGR3a genotype influence the response to rituximab in immune thrombocytopenia.

Rituximab is widely used in autoimmune diseases including immune thrombocytopenia (ITP), although the mechanism of effect remains unclear. This study **(Cooper N. et al. Br J Haematol 2012; 158(4): 539-47)** describes the effects of rituximab on platelet-associated antibodies (PA-APAs), B and T cell counts and clonality (IGHV and TRG@ gene rearrangements), FCGR3A (FcγRIIIa) and FCGR2A (FcγRIIa) polymorphisms and correlation to anti-CD40 ligand (CD40L) response. PA-APA levels fell more frequently in responders (6/8) than in non-responders (2/10: P = 0•08-0•15). Two responders had no PA-APAs. Two non-responders with a fall in PA-APAs had very high CD8 levels. One non-responder had a B cell clone, one responder and one non-responder had a T cell clone. 15/16 patients had the same responses to rituximab and antiCD40L. Patients with FCGR3A V/V polymorphisms were more likely to respond to rituximab (P = 0•03). In summary, the fall in PA-APAs in responders confirms the humoural effect of rituximab. Failure to respond in patients with very high CD8 levels, despite PA-APA fall indicates a role for T cell-mediated platelet/megakaryocyte destruction. Concordance of response to anti-CD40L suggests autoantibody-producing cells are under T cell control. Finally, the effect of FCGR polymorphisms on response confirms the importance of FCGR-mediated depletion of B cells in autoimmunity. This has implications on the pathology of ITP as well as the immunological effect of B cell depletion.

E. Toubi

第54回日本老年医学会学術集会記録
〈Aging Science Forum：エイジングサイエンスと抗加齢医学・医療〉

2. 筋肉と神経のシナプスの老化（サルコペニア）の基礎研究

重本 和宏　森 秀一　福永 大地　宮崎 剛

Key words：サルコペニア，運動神経細胞，神経筋シナプス，筋線維

（日老医誌 2013；50：349-351）

はじめに

超高齢社会を迎え，有効な介護予防を達成するために科学的根拠に基づく診断・予防・治療法の開発が求められている．特にサルコペニア（加齢性筋肉減少症）は，リハビリの有効性や介護予防を可能にする早期診断など，臨床的に有効な対策は全く確立されていないのが現状であることから，そのメカニズム解明は急務の課題である．臨床現場における診断基準としては，筋力，筋肉量，身体能力の3つで測定評価することになるが[1]，基礎研究を遂行するためにはサルコペニアの定義を，病理学的観点から再構築する必要がある．

サルコペニアと病理学的変化

筋肉の老化に関する研究は，これまでも多く発表されている．しかしながら，サルコペニアのメカニズムを本質的に解明するに至っていないのが現状である．サルコペニアの原因は多因子によるものであり，それらを明らかにして実験的にサルコペニアを再現するのは極めて困難である．そこで，サルコペニアと並んで要介護の原因となる認知症の研究経過を参考に考慮すると，そのメカニズムや治療法・予防法の研究は厳密な神経病理学的な定義を基にしておこなわれていることがわかる．様々な認知症の発症メカニズム，診断法や治療法の効果判定は，症状による分類や臨床的な検査項目だけでなく病理学的根拠に基づいて評価することが必要とされている．同様にサルコペニアにおいても，病理学的指標に基づいた基礎研究が必要である．つまり，筋肉の量的および質的変化に加えて，運動神経細胞，筋と運動神経細胞のつなぎ目である神経筋シナプスも対象としてサルコペニアの病理学的な変化を関連づけて明確にしておく必要がある．

運動神経細胞の変化

では，老化により運動神経細胞はどのような病理学的変化を示すのであろうか？　加齢による運動神経細胞死に関するデーターは極めて少ないが，まずヒトの運動神経細胞数の変化については，1977年にイギリスから発表されたデーターがある[2]．死亡時に運動機能は正常に保たれていた13歳から95歳までの47人の腰仙髄の一定領域内の運動神経細胞を数えたところ，60歳を境にして急速に運動神経細胞数が減少していることを示した（図1）．また図2に示すように，近交系ラットの生後20カ月から腓腹筋を支配する運動神経細胞数が減少することが報告されている[3]．ヒトおよびラットのデーターは共に，何らかの疾患が原因ではなく加齢に起因する運動神経細胞死と解釈してよいだろう．超高齢社会を迎えた現在でも，ヒトの脊髄の運動神経細胞数が加齢に伴って一様に減少するかどうか，あるいは個人差が大きくなっている可能性についても興味が持たれる．また，実験動物を使ったサルコペニアの研究を行うときは，対象とする加齢動物の月齢により運動神経細胞数が減少することを考慮する必要があることを示している．加えて，運動神経細胞は発生期において，異なる遺伝子発現制御により分化する異なる細胞集団から構成されているが，その点を考慮せずに単純に細胞死をカウントするだけではサルコペニアの病態を見逃す可能性が考えられる．また，運動神経細胞が減少するのは細胞死が原因であると考えられるが，その直接原因について明らかにする必要がある．加齢による脊髄の運動神経細胞の脱落はサルコペニアと因果関係があると予想されるが，筋力低下や筋萎縮など臨床症状の出現と運動神経細胞数との関係（閾値）について，よくわかっていないのが現状である．

Aging research on muscle and motor neuron
Kazuhiro Shigemoto, Syuuichi Mori, Taichi Fukunaga, Tsuyoshi Miyazaki：東京都健康長寿医療センター研究所老年病態研究チーム運動器医学

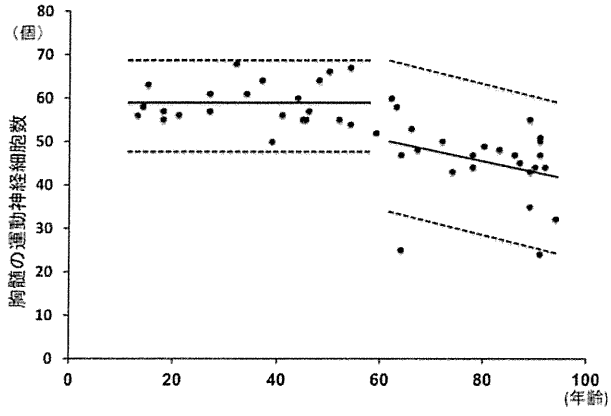

図1　加齢による脊髄の運動神経細胞数の減少（ヒト）
（文献2の図を改変）

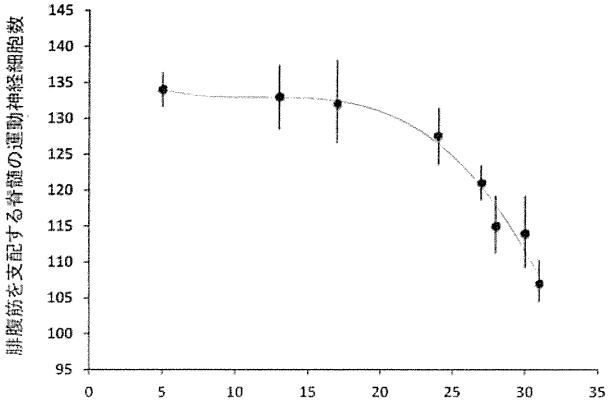

図2　加齢による脊髄の運動神経細胞数の減少（ラット）
（文献3の図を改変）

いっぽう，加齢による運動神経細胞数の減少に伴う詳細な組織像についての情報は，ほとんど欠如している．超高齢社会を迎えて，運動神経細胞の脱落が主原因とされているALS（筋萎縮性側索硬化症）の患者が増加しているが，一部の非定型のALSはもともと診断が難しく，サルコペニアとの鑑別が問題となりそうである．そして，ALSで見られるような典型的な病理組織像が，臨床的にサルコペニアと診断された患者の脊髄でも観察されるかどうか，またサルコペニアと認知症と接点があるかどうかについても，今後の病理学的な検討が必要である．

神経筋シナプスの形態変化

次に運動神経と筋のつなぎ目，神経筋シナプスは加齢によりどのような形態変化を示すであろうか？　ヒトの組織像に関する報告は非常に少ないが，図3に示すように，加齢によりシナプスの襞の減少が顕著となる[4]．マウスやラットでも加齢によりシナプス襞が減少し，さらにシナプスの形態が断片化，神経終末の縮小，神経終末のsprouting，部分的あるいは完全な脱神経支配が観察される（図4）．マウスでは，体幹，後肢や頸部の神経筋シナプスは，加齢による形態変化が顕著に観察され，いっぽう外眼筋や外肛門括約筋では形態が保たれているが，ヒトについてこれと同じように筋の加齢変化がおきるかどうかは不明である．このシナプス形態の変化は，神経伝達の効率を下げて筋力低下や筋萎縮の原因になる．実際に，重症筋無力症で自己抗体がシナプス形態および機能の維持機構が著しく障害すると筋萎縮を誘導する[5][6]．シナプスの維持は，シナプスを介した運動神経終末と筋の相互作用により保たれている．つまり，筋あるいは運動神経細胞のどちらかに障害が生じるとシナプスの形態と機能が障害される．シナプスの筋肉側に発現するMuSK（muscle-specific kinase）が，このシナプスの相互維持に重要な役割を果たしていることから，MuSKの上流および下流の分子機構が加齢により変化することが予想される[6]．

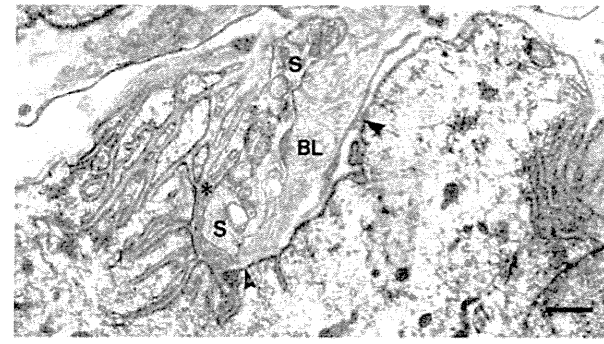

図3　75歳男性の神経筋シナプス形態
シナプス襞が減少している．（文献4の図を改変）

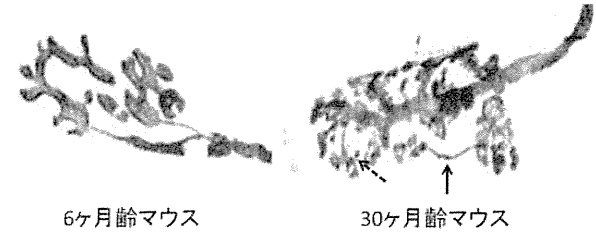

図4　加齢に伴うマウス神経筋シナプスの形態変化
アセチルコリン受容体凝集の散乱（点線矢印），運動神経終末のsprouting（実線矢印）．

神経筋シナプスは可塑性があり再生が可能である．そして，シナプス形態の加齢変化をカロリー制限や運動により予防できることが，マウスを使った実験で示されている[7]．カロリー制限では全身のシナプスの形態が若返

るのに対して，運動刺激は与えた筋のシナプスだけを改善した．また，カロリー制限の方が運動刺激よりも改善度が良かった．マウスのカロリー制限は，生後4カ月から始めて24カ月齢まで行っているが，老年期に開始して有効かどうかは不明である．運動刺激は22カ月齢のマウスに対して1カ月間与えることで有効性が確認された．この時期の高齢マウスは，まだ神経筋シナプスの可塑性が維持されていることを示しているが，ヒトも含めて加齢がさらに進んでもシナプスの可塑性が保たれるかどうか，また運動神経細胞数の減少との関連については今後の検討が必要である．

筋線維の変化

サルコペニアにおける筋の病理学的特徴については，筋線維数および断面積の減少だけでなく筋線維の質的変化が起きることがわかっている．筋は速筋と遅筋の筋線維タイプで構成され，それぞれ収縮力およびそれに伴うエネルギー産生の特性が異なっている．筋の可塑的な質的変化は運動などによってもおきるが，加齢によりその可塑性を失う．上述の運動神経細胞および神経筋シナプスの加齢変化と筋線維タイプの可塑性変化は密接な因果関係があると予想される[8]．

ま と め

サルコペニアのメカニズムを解明するために様々なアプローチが試みられているが，導かれた仮説が病理学的変化を説明できるかどうか動物モデルを使って検証する必要がある．高齢動物がサルコペニアを代表するモデルとなるかどうかについて議論はあるが，同じ論争は他疾患のモデル動物を使った研究についても必ずおきる．サルコペニアの有効な対策は認知症と同じく早期診断・介入が必要であるが，そのためには加齢に伴う運動神経細胞の脱落，神経筋シナプスおよび筋組織の可塑的な維持メカニズムについて，動物を使った基礎研究が必要である．さらに，壮年期を過ぎて老齢期のある時期に，筋，運動神経細胞，シナプスそれぞれの維持機構に関わるメカニズムの可塑性を喪失する臨界の存在が予想され，加齢動物はそれを明らかにするために重要なツールとなるであろう．

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筋肉の老化

重本 和宏[*1] 福永 大地[*2] 森 秀一[*3]

筋肉の老化，すなわちサルコペニアについて最近注目されている。臨床や基礎の様々な研究分野で，その診断・予防法やメカニズムの研究がなされている。筆者は，サルコペニアに伴う関連組織（運動神経細胞，シナプス，筋線維，サテライト細胞）の特徴的な病理組織像は，サルコペニア研究を展開する上で重要な指標となると考えており，最近の知見を踏まえて本稿で概説する。

Aging and Bio-motor function.

Aging of muscle.

Tokyo Metropolitan Institute of Gerontology, Japan.

Kazuhiro Shigemoto, Taichi Fukunaga, Shyuuichi Mori

Sarcopenia has attracted attention recently in various fields of clinical and basic research to understand the mechanisms and developing new methods for diagnosis and prevention. We review pathological features in motor neurons, synapses, muscle fibers, satellite cells associated with sarcopenia based on recent findings. The author believes that the pathological evidence comes to an important measure in the development of research on sarcopenia.

はじめに

サルコペニア（加齢性筋肉減少症）は臨床的には，筋力，筋量，身体能力（歩行など）の3つの測定可能な指標で定義・診断される。しかし，少なくとも基礎老化学の立場から見れば，サルコペニアのメカニズムを明らかにするためには病理学的な基準も導入する必要がある。骨格筋は，筋線維だけでなく由来の異なる多様な細胞から構成され複雑な組織である。また骨格筋の維持には，神経筋シナプスを介して運動神経細胞との相互作用が重要な役割を果たしている。

本稿では，老化による筋力低下と筋萎縮に伴う

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筋・運動神経細胞の病理学的変化について概説する。

サルコペニアの臨床的定義と診断

超高齢社会を既に迎え，骨格筋量の進行的な低下に伴う機能低下，すなわちサルコペニア（加齢性筋肉減少症）は認知症と並んで高齢者のactivity of daily living (ADL) と quality of life (QOL) を損なう主要な原因となることから，その早期診断や有効な介護予防対策は急務の課題である。サルコペニアの有病率は60～70歳で5～13%，80歳以上の高齢者の有病率は11～50%との報告がある[1]。サルコペニアの有病率は世界的にも増加しており，高齢者の健康と福祉の観点からサルコペニアについて臨床だけでなく様々な分野から研究を進める必要がある[2]。サルコペニアの臨床定義および臨床基準として，筋力，筋量と身体能力の3つの指標を定量的に測定して評価することが，欧州の統一見解として2010年に発表された[3]。2012年には厚生労働省研究班から，その監訳とわが国の現状から鑑みた欧州基準に対する見解がQ＆Aとして発表されている[4]。日本人の体格に合わせてカットオフ値をどう定めるか，異なる臨床研究のデータ間で比較検討が可能な測定方法を統一できるかどうか，今後の課題である。

サルコペニアの分類とメカニズム

サルコペニアは原因により，「一次性」（加齢性）と一つ以上の原因が明らかな「二次性」に臨床的に分類される（表）[3,4]。しかし，サルコペニアのメカニズムを解明するには臨床的な分類以外の指標も必要である。認知症の研究では，そのメカニズムや治療法・予防法の研究は厳密な神経病理学的な定義による臨床診断を基準にしている。同様に，サルコペニアのメカニズムを解明するためには，病理学的指標に基づいた基礎研究が必要である。

筋肉の老化のメカニズムに関する多くの研究は，「一次性」（加齢性）のサルコペニアを対象としているが，そのメカニズムを本質的に理解するには至っていない。サルコペニアは，様々な要因による長時間の相互作用の結果によるものであり，ヒトを対象とした研究には制限がある。臨床的には，高齢者は「一次性」のサルコペニアに加えて，生活習慣病など種々の疾患に伴う原因を高頻度で有していることから，それぞれの原因と病理学的変化を対応させることは非常に困難である。そのため，老化モデル動物は老化に伴う「一次性」サルコペニアのメカニズムを知るための，重要なツールとなっている。これまで，サルコペニアに関連する病理学的変化として，運動神経細胞数の

表　原因に基づくサルコペニアの分類

サルコペニアは原因により，「一次性」（加齢性）と一つ以上の原因が明らかな「二次性」に臨床的に分類される。

一次性サルコペニア 　加齢性サルコペニア	加齢以外の原因が明らかでないもの
二次性サルコペニア 　活動に関連するサルコペニア 　疾患に関連するサルコペニア 　栄養に関係するサルコペニア	 寝たきり，不活発な生活様式，（生活）失調や無重力状態が原因となり得るもの 重症臓器不全（心臓，肺，肝臓，腎臓，脳），炎症性疾患，悪性腫瘍，内分泌疾患 吸収不良，消化管疾患，食欲不振を起こす薬剤使用などに伴う，摂取エネルギーおよび/またはタンパク質の摂取量不足に起因するもの

（文献3，4より改変）

ADL：activity of daily living，QOL：quality of life