Geriatrics Gerontology INTERNATIONAL 1st Impact Factor in June 2010 Medline Indexed

Geriatr Gerontol Int 2010; **10 (Suppl. 1):** *S137–S147*

Muscle weakness and neuromuscular junctions in aging and disease

Kazuhiro Shigemoto,[1] Sachiho Kubo,[1] Syuuichi Mori,[1] Shigeru Yamada,[1] Takuyu Akiyoshi[1,2] and Tsuyoshi Miyazaki[1]

[1]*Research Team for Molecular Biomarkers, Tokyo Metropolitan Institute of Gerontology, and* [2]*Department of Geriatric Medicine, Tokyo University School of Medicine, Itabashi, Tokyo, Japan*

A critical issue in today's super-aging society is the need to reduce the burden of family care while continuing to make our medical institutions supportive. A rapidly emerging, major health concern is the debilitating effect of muscle weakness and atrophy from aging, termed sarcopenia; however, the molecular basis of this condition is not well understood. Our research aim is to elucidate the molecular mechanisms of age-related muscle atrophy and to devise new measures for preventing and treating this disability. A promising treatment for muscle atrophy is the promotion of muscle regeneration by recruiting stem cells into the targeted region. The first requirement is to understand how the motor system, which consists of muscles and motoneurons, is maintained to accomplish that goal. Recent studies in the field of neuroscience have focused on neuromuscular junctions (NMJ), which play important roles in the maintenance of both motor nerves and muscle fibers. Signaling between muscles and motoneurons at NMJ supports interactions within the motor system. To understand the mechanisms involved, we focus our research on the pathogenic processes underlying neuromuscular diseases. The well-known autoimmune disease, myasthenia gravis (MG), serves as a model not only for tracking the pathogenesis and treatment outcomes of all autoimmune diseases, but also for understanding synaptic functions in maintaining the motor system. Here, we describe recent insights into the molecular mechanisms required for the maintenance of NMJ and the related causes of muscle atrophy. **Geriatr Gerontol Int 2010; 10 (Suppl. 1): S137–S147.**

Keywords: muscle-specific kinase, myasthenia gravis, neuromuscular junction, sarcopenia.

Neuromuscular junctions

Neuromuscular junctions (NMJ), which are structures located between motor terminals and muscles, are the sites of synapses between motor nerves and muscle fibers. At the anterior horn of the spinal cord and brainstem, skeletal muscle fibers are innervated by large motor neurons. The terminal arborization of each α-motor neuron is situated in a shallow depression of the muscle cell membrane, which is invaginated further into deep and regular folds, termed postjunctional folds (Fig. 1). The motor nerve terminal is specialized for neurotransmitter (acetylcholine; ACh) release. Synaptic vesicles containing ACh cluster adjacent to specialized structures of the presynaptic membrane, called active zones. The active zones are aligned precisely with mouths of the post-junctional folds. ACh receptors (AChR) are highly concentrated, with a density of about 12 000 receptors per μm^2, at the post-junctional membrane nearest to the fold's peak (Fig. 1). When the nerve action potential reaches the terminal, depolarization opens voltage-gated Ca^+ channels on the presynaptic membrane. This allows a Ca^+ influx that triggers the

Accepted for publication 20 November 2009.

Correspondence: Dr Kazuhiro Shigemoto MD PhD, Tokyo Metropolitan Institute of Gerontology, Research Team for Molecular Biomarkers, Sakaecho 35-2, Itabashi-ku, Tokyo 173-0015, Japan. Email: kazshige@tmig.or.jp

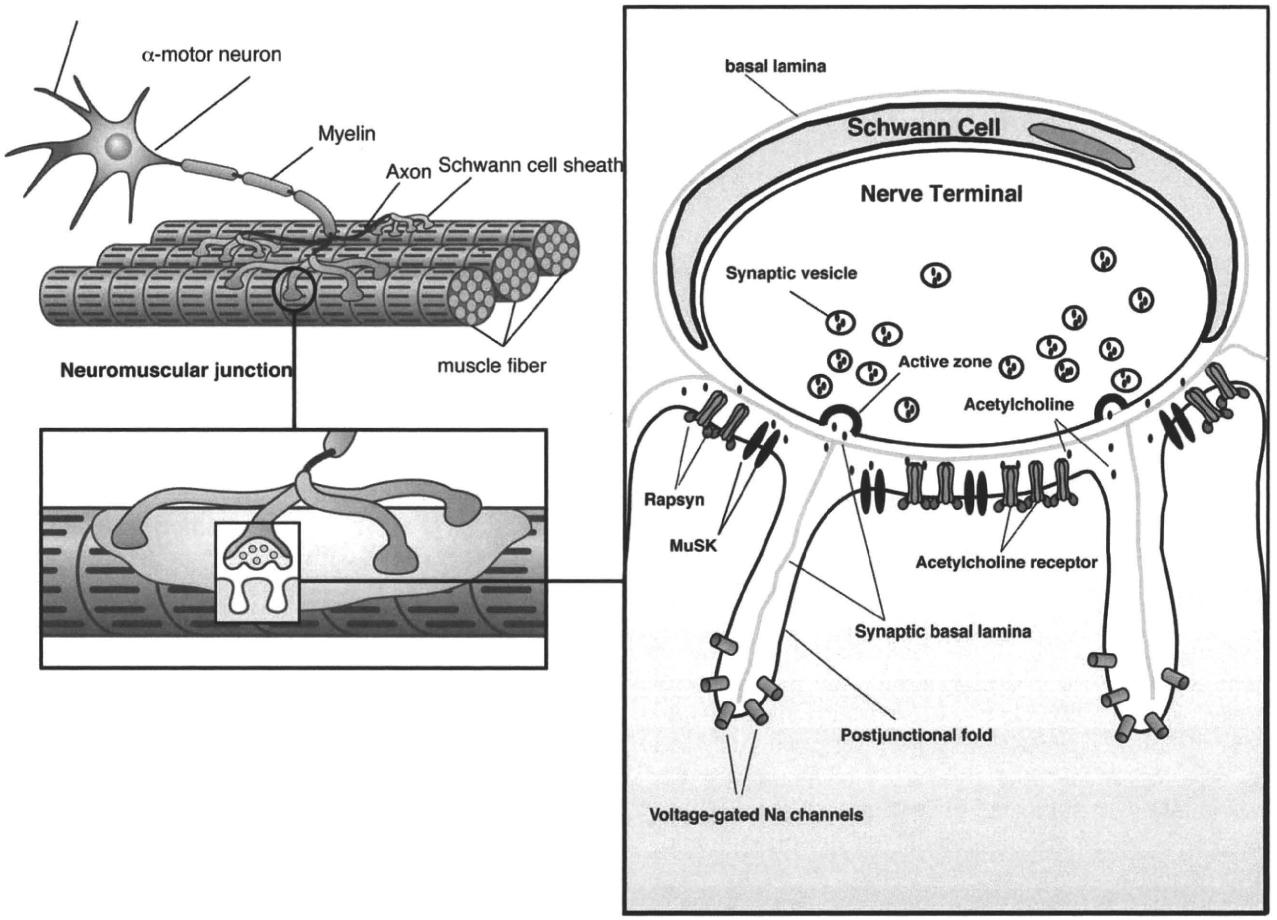

Figure 1 Structure and molecular architecture of the neuromuscular junctions (NMJ). Drawings show progressive enlargement of segments of a NMJ. The presynaptic terminals consist of multiple swellings called synaptic terminals covered by a thin layer of Schwann cells. The nerve terminal occupies a shallow gutter in the muscle fiber and is capped by processes of Schwann cells. Acetylcholine (ACh) from 150 to 200 vesicles is released from the active zones in the nerve terminal, which directly oppose junctional folds in the postsynaptic membrane. The terminals are separated from the postsynaptic cell by the synaptic cleft, which is about 50 nm wide. Acetylcholine receptors, muscle-specific kinase (MuSK) and rapsyn concentrated at the peaks of postsynaptic folds are shown, with their subcellular localizations indicated by bars. Voltage gated sodium channels are localized in the depths of postsynaptic folds.

fusion of synaptic vesicles with the presynaptic membrane and the release of ACh. The post-synaptic membrane responds rapidly and dependably to ACh released from the overlying active zones in the nerve terminal. AChR, by binding ACh, become transiently permeable to both Na^+ and K^+, then opening the associated voltage-gated ion channels, which contribute to the action potential and muscle contraction. The synaptic cleft between nerve terminals and postsynaptic membrane is approximately 50 nm wide. A layer of connective tissue called basal lamina (basement membrane) sheaths each muscle fiber, passes through the synaptic clef and extends into the junctional folds. Both the presynaptic terminal and the muscle fiber secrete molecules including collagen IV, laminin, ectactin and heparan sulfate proteoglycans to the basal lamina. However, synaptic portions of the basal lamina contain their distinctive isoform composition separate from that of the extrasynaptic portions. Synaptic basal lamina also contain the enzyme acetylcholinesterase, which quickly inactivates the ACh released from the presynaptic terminal by hydrolyzing it to acetate and choline. Concentrations of released ACh in the synaptic cleft decrease rapidly by diffusion and interaction with acetylcholinesterase, upon which the neuromuscular transmission terminates.

Myasthenia gravis and autoantibodies to AChR

Myasthenia gravis (MG) is a rare neuromuscular disease, but a well-recognized disorder because of such characteristic clinical features as ptosis with fluctuating general fatigue and muscle weakness that worsens with

repeated activity,[1,2] but tends to improve with rest. Ptosis and diplogia occur early in the majority of these patients. With passing time, when the bulbar and respiratory muscles deteriorate, the disease becomes life-threatening so that intubation with mechanical ventilation is required. Approximately 80% of patients with MG have autoantibodies against AChR.[1,2] In 1973, Patrick and Lindstrom provided the first evidence indicating the pathogenicity of AChR antibodies in a model of experimentally induced MG.[3] Thereafter, a number of studies showed the pathogenic roles of AChR antibodies in causing structural and functional damage of the NMJ, but no such autoantigens could be identified in ~20% of these MG patients.[4] However, even patients who did not have AChR antibodies responded to immunotherapies, and their serum antibodies transferred a defect in neuromuscular transmission to mice, indicating that autoantibodies against NMJ can induce the muscle weakness.

Previously, studies on the mechanism(s) of synaptic transmission at the NMJ had facilitated understanding of how antibodies to AChR induce the pathogenicity typical of MG.[1,2] Effective neuromuscular transmission depends on numerous interactions between ACh and its receptor, AChR, and the failure of neuromuscular transmission results in myasthenic weakness and fatigue. To evoke action potential for the contraction of muscle fibers, a large enough number of AChR must be present at postsynaptic membranes. In 1973, Fambrough *et al.* found an abnormal decrease in the number of AChR at postsynaptic membranes of the NMJ of patients with MG.[5] Others showed that AChR antibodies affect neuromuscular transmission by three main mechanisms: (i) complement-mediated lysis of post-synaptic membrane by binding and activation of complement at the NMJ; (ii) accelerated degradation of AChR molecules cross-linked by antibodies (antigenic modulation); and (iii) functional AChR block by antibodies. The predominant pathogenicity is caused by the complement-mediated mechanisms,[6] but all three mechanisms tend to reduce the number of available AChR and, thereby, decrease neuromuscular transmission between motor nerve endings and postsynaptic membranes. Therefore, an individual nerve impulse cannot generate enough postsynaptic depolarization to achieve the crucial firing threshold required for opening of sufficient voltage-gated sodium channels to initiate an action potential in the muscle fiber.[7]

Antibodies to muscle-specific kinase in myasthenia gravis (MG) patients

For the last three decades, causative autoantibodies other than those to AChR have been sought in MG patients but have eluded identification despite extensive research efforts.[1,2] In 2001, Hoch *et al.* found autoantibodies against muscle-specific kinase (MuSK) in a proportion of patients with generalized MG.[4] MuSK is essential during the development of NMJ, when it organizes fetal AChR clustering at the postsynaptic membrane. Subsequently, in mature NMJ, MuSK is expressed predominantly at the postsynaptic membrane. Studies by Vincent *et al.* showed that the frequency of MuSK antibodies in "seronegative MG patients," that is those who lack autoantibodies to AChR, varied from 4 to 50%.[4,8–11] Ohta *et al.* detected MuSK antibodies in approximately 30% of seronegative MG patients but not in any MG patients with AChR antibodies (seropositive MG) or other autoimmune diseases.[12–14] The clinical features of patients with MG and MuSK antibodies are distinctive. These individuals often suffer from a severe bulbar dysfunction that is difficult to resolve with immunosuppressive and immunomodulatory treatments, and muscular atrophy of facial and tongue muscles is common.[14,15] The response to acetylcholine esterase inhibitors is generally unsatisfactory with the risk of worsening symptoms, especially when starting treatment in patients with bulbar symptoms or an impending respiratory crisis.[16] Thymectomy does not alleviate the symptoms.[14] In short-term therapy, patients with MuSK-positive MG respond as well to plasma exchange and intravenous immunoglobulin as those with AChR seropositive MG.[14] Even so, those patients whose neck and shoulder muscles are affected often experience respiratory weakness.[15] MG in which weakness is limited to the ocular muscle is not frequent but does occur.[15]

A number of clinical studies showed that MuSK MG constitutes a distinct subclass of the disease.[8–10,15] The reason is that many patients with MuSK antibodies develop severe muscle weakness and eventual atrophy, which is less common in patients with AChR seropositive MG, and the former respond differently to therapy than persons in the latter group. After the identification of MuSK antibodies in an MG patient, laboratory testing is now required to confirm the diagnosis of MG, to seek AChR antibodies and to formulate the clinical treatment.

MuSK functions in neuromuscular junctions (NMJ)

MuSK plays multiple roles in clustering AChR during development of the postsynaptic membranes of NMJ.[17,18] Contact of the motor-nerve growth cone with the muscle induces a narrow, distinct endplate zone in the mid-muscle that is marked by a high density of AChR clustering. In this step, agrin released from motoneurons activates MuSK and redistributes AChR clusters to synaptic sites.[18,19] However, agrin does not bind MuSK, and additional components are required to activate MuSK.[17,19] Recent studies showed that Lrp4,

a member of the LDLR family, is a receptor of agrin, forms a complex with MuSK and mediates MuSK activation by agrin.[20,21] Intriguingly, MuSK is also required for organizing a primary synaptic scaffold to create the post-synaptic membrane.[18] Prior to muscle innervation, AChR clusters form at the central regions of muscle fibers, creating an endplate zone that is somewhat broader than that in innervated muscle. Thus, MuSK is required for pre-patterning of AChR clustering in the absence of motor innervation. However, establishing a scenario for MuSK's participation in the process is somewhat complicated. For example, an element other than agrin might activate MuSK and trigger the postsynaptic specialization at NMJ. Simultaneously or alternatively, MuSK could act as a primary scaffold molecule without activation. The listed pleiotropic roles of MuSK in AChR clustering at developmental NMJ could also be required for the maintenance of mature NMJ.[22,23] Studies carried out *in vivo* have shown that synaptic AChR intermingle among themselves completely over a period of ~4 days and that many extra-synaptic AChR are incorporated into the synapse at the mature NMJ, although the synaptic membrane in adult muscle appears macroscopically to be stable.[24] Therefore, the mechanisms at play during AChR clustering in developing NMJ are also required in mature NMJ where postsynaptic complexes including those with AChR and MuSK are dynamically turning over for the maintenance of muscle function.

Do MuSK antibodies cause MG?

In contrast to the well-accepted mechanisms by which AChR antibodies function in MG, the pathogenic role of MuSK antibodies has been unclear.[25] First, no significant loss of AChR at NMJ was observed in biopsies from biceps brachii muscles of MuSK-positive patients with MG.[26] Second, MuSK antibodies are mainly in the IgG4 subclass, which does not activate complement[14] and complement-mediated damage to postsynaptic membranes, is considered a major source of pathogenicity in MG patients with AChR antibodies. Third, no research results have shown that passive transfer of MuSK serum from MG patients generates the equivalent disease in mice. Fourth, no experimental animal model of myasthenia gravis (EAMG) induced by immunization of MuSK protein has been developed. However MuSK antibodies from MG patients can inhibit MuSK functions *in vitro*.[4]

The pathogenicity of AChR antibodies was simulated experimentally by the induction of muscle weakness and development of paralysis in rabbits immunized with AChR protein purified from the electric eel.[3] This AChR protein induced the production of antibodies that cross-reacted with rabbit AChR at the NMJ. The flaccid paralysis that followed and electrophysiological studies

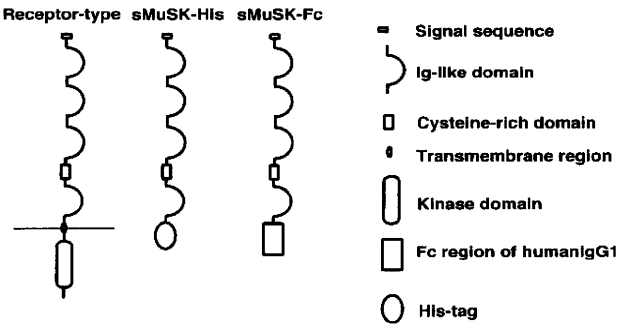

Figure 2 Schematic representation of the muscle-specific kinase (MuSK) domain structure and expression of secretory MuSK proteins in COS-7 cells. The domain structures of recombinant secretory MuSK protein (MuSK-His and MuSK-Fc) and receptor-type MuSK are shown. The whole coding region of the MuSK extracellular domain was fused with the His-tag or Fc region of human IgG1 as shown.

of these animals provided a model that resembled the MG of humans. Therefore, the demonstration of experimental autoimmune MG in animals induced by MuSK antibodies was essential for proving their pathogenicity and investigating their mechanisms of eliciting MG.

In 2006, we found that immunization of rabbits with MuSK ectodomain caused myasthenic weakness and produced electromyographic findings that were compatible with a diagnosis of MG,[23] as shown earlier by Patrick and Lindstrom.[3] The extracellular segment of MuSK comprised five distinct domains, that is four immunoglobulin-like domains and one cysteine-rich region (Fig. 2). The fusion protein expression constructs, which consisted of mouse MuSK ectodomain with the Fc region of human IgG1 or His-tag, were generated and transfected into COS-7 cells. The secreted recombinant MuSK-Fc and MuSK-His proteins were purified by using protein-A Sepharose and histidine affinity columns, respectively. New Zealand White rabbits were then immunized with 100–400 μg of purified MuSK recombinant protein. After three to four injections of MuSK protein, all six rabbits manifested flaccid paralysis (Fig. 3a). Sera from the paretic rabbits contained a high titer of MuSK antibodies that reacted specifically with MuSK molecules on the surfaces of C2C12 myotubes as observed in sera from MG patients who were positive for MuSK antibodies.[23] Histological studies of the muscle tissues from the paretic rabbits, which had manifested severe exhaustion, showed alterations in muscle fibers ranging from subtle to angular atrophy intermingled with normal muscle tissue (Fig. 3b). The histological changes typical of atrophied muscle fibers can result from MG, reduced mechanical ability or cachexia. In repetitive electromyograms from one of these paretic rabbits, the retroauricular branch of facial nerve was stimulated at 20 Hz, and recordings were taken from adjacent retroauricular muscle (Fig. 3c). The

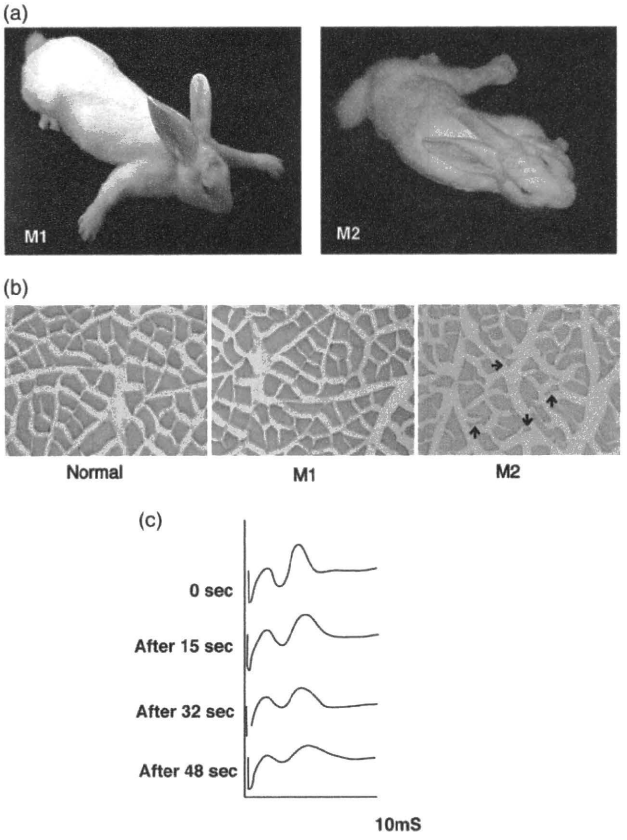

Figure 3 Rabbits manifest myasthenia gravis (MG)-like paresis after immunization with muscle-specific kinase (MuSK) protein. (a) Two rabbits representative of four animals with positive outcomes developed myasthenic weakness after immunization with the recombinant MuSK protein. After three injections of MuSK protein, M1 and M2 rabbits manifested flaccid weakness within 3 and 9 weeks, respectively. M2 rabbit developed severe exhaustion with muscle weakness. (b) Cross-sections from the soleus muscles of two paretic rabbits (M1 and M2) and a normal rabbit (Normal) were stained with hematoxylin–eosin. Muscle fibers in M1 paretic rabbit showed only subtle changes in shape and size, whereas atrophy of muscles fibers in M2 paretic rabbit was observed as small angular fibers (indicated by arrows; bar, 50 μm.) (c) Electromyograms recorded from M1 paretic rabbit. The retro-auricular branch of the facial nerve was continuously stimulated with constant square-wave pulses of 0.1 msec at 20 Hz delivered by a current stimulator, and the compound muscle action potential (CMAP; second peak observed on the oscilloscope screen recorded at the indicated time-points during stimulation) showed a decremental pattern, consistent with MG. Reproduced from *J Clin Invest* 2006; 116: 1016–1024 with permission. © 2009 The American Society for Clinical Investigation.

compound muscle action potential (CMAP) showed a decremental pattern, consistent with MG. However, injections of acetylcholine esterase inhibitor did not significantly reverse either the CMAP defect or the paralytic symptoms. Importantly, induction of myasthenia by

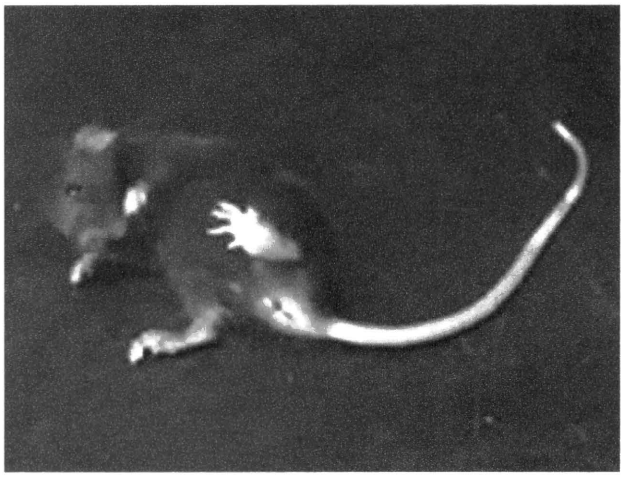

Figure 4 Manifestations of myasthenia gravis after injection of purified muscle-specific kinase proteins in a mouse.

MuSK antibodies is not confined to the rabbit, because we and others also produced myasthenia in mice by injection of MuSK protein (Fig. 4).[27,28]

How do antibodies to MuSK cause MG?

We have provided the first piece of evidence that active immunization with MuSK protein reproduces the MG-like disease in animals.[23,28] Next, we focused on how MuSK antibodies cause MG. The pathogenic role of MuSK antibodies in MG has been questioned, because the number of AChR is not reduced and complement is not deposited at the NMJ of biceps brachii muscles from MuSK-positive patients with MG.[26] The mechanisms used by AChR antibodies to cause MG are well delineated,[1,2] but those mechanisms simply do not apply to MG associated with MuSK antibodies. MuSK antibodies have been identified as predominantly IgG4, which does not activate complement. However, antibodies binding to MuSK could accelerate the degradation of MuSK molecules (antigenic modulation) and/or inhibit MuSK functions directly. MuSK is essential for AChR clustering at the developing NMJ, and its deficiency might lead to the complete loss of junctional ultrastructure.[22,29] Further, MuSK might also play important roles in the maintenance of AChR clustering and the structure of mature NMJ. To show precisely how MuSK antibodies participate in MG, unraveling the way in which MuSK acts at mature NMJ is necessary.

To elucidate the mechanisms of AChR clustering at NMJ, numerous studies were carried out using cultured C2C12 myotubes (Fig. 5). Agrin induces clustering of AChR in C2C12 myotubes after autophosphorylation by MuSK. *In vivo*, this event represents a major cascade of

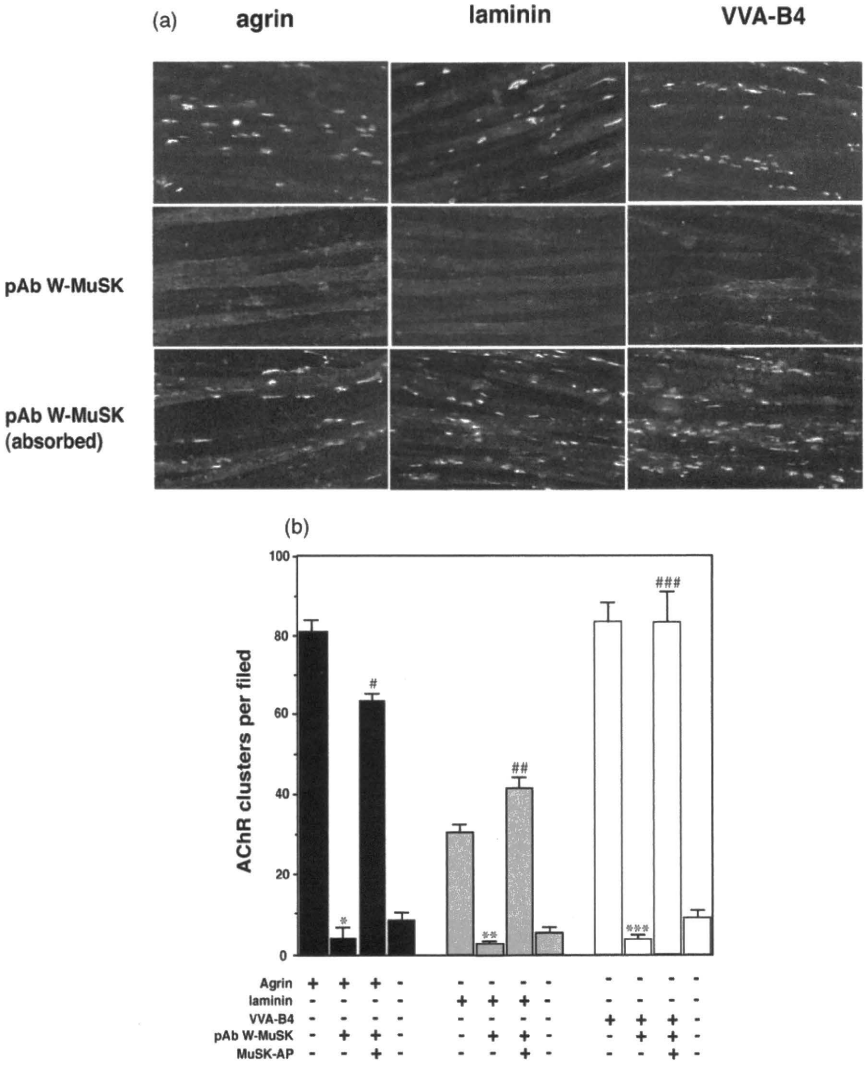

Figure 5 Inhibition of agrin-induced and agrin-independent acetylcholine receptors (AChR) clustering by muscle-specific kinase (MuSK) antibodies. (a) C2C12 cells were treated with agrin, laminin-1 or VVA-B4. AChR clusters were stained with rhodamine-conjugated BTX. AChR clustering induced by agrin, laminin-1 and VVA-B4 was inhibited in the presence of MuSK antibodies. This inhibition was blocked by absorption of the MuSK antibodies with MuSK-AP before treatment of the cells (bar, 20 μm). (b) Quantification of the inhibitory activity of the MuSK antibodies confirmed that they significantly inhibited agrin-, laminin-1– and VVA-B4-induced AChR clustering. Preabsorption of the MuSK antibodies with MuSK-AP significantly blocked inhibition. Values represent means ± SEM of 10–15 fields for each of the two experiments per treatment. $^{*,**,***}P < 0.01$ versus similar treatment without MuSK antibodies; $^{\#,\#\#,\#\#\#}P < 0.01$ versus similar treatment without preabsorption; ANOVA. *J. Clin. Invest.* 2006; **116**: 1016–1024. Copyright 2009 The American Society for Clinical Investigation.

AChR clustering at the NMJ after innervation by motoneurons. Laminin-1 and the *N*-acetylgalactosamine (GalNAc)-specific lectin *Vicia villosa* agglutinin (VVA-B4) also induce AChR clustering on C2C12 myotubes, without activation of MuSK. Neither the receptor nor the activation mechanisms of AChR clustering induced by agrin-independent inducers has been identified with certainty. However, these mechanisms might also be important for the formation and maintenance of NMJ, the latter through agrin-independent pathways as shown by genetic studies.[23]

In a previous study, Hoch *et al.* observed that the MuSK antibodies of MG patients inhibited agrin-induced AChR clustering in C2C12 myotubes.[4] We also found that agrin-induced clustering of AChR was strongly blocked in the presence of MuSK antibodies, whereas absorption of the antibodies with purified MuSK products prevented this blocking effect as shown in Figure 5.[23] These results showed that MuSK antibodies effectively inhibited the formation of agrin-induced AChR clustering. Intriguingly, the monovalent Fab fragments of MuSK antibodies from rabbits with

experimental autoimmune MG also inhibited AChR clustering by agrin on C2C12 cells, indicating that complement-mediated mechanisms are not necessarily required for such inhibition (unpubl. data). We also noted that MuSK-specific antibodies strongly inhibited AChR clustering induced by all known agrin-independent pathways as well as by agrin itself (Fig. 5).[23]

We then examined the reduced expression of AChR at NMJ in soleus muscles of paretic and normal rabbits by using fluorescence microscopy after applying a rhodamine-conjugated AChR agonist, α-BTX (Fig. 6). The use of a digital camera and staining with rhodamine-conjugated α-BTX enabled us to record the size and optical densities of AChR clusters. The resulting images were measured by using NIH image analysis software.[23] The areas and intensity of AChR fluorescence in muscles of these paretic rabbits were significantly reduced compared with those in normal rabbits. In addition, the structure of NMJ in our paretic rabbits as well as the size and branching of the motor terminals were significantly reduced. Electron microscopic observations of NMJ in rabbits with EAMG induced by injection of MuSK protein showed a significant loss of complexity in the convoluted synaptic folds but no destruction. A particularly important observation was that the EAMG model cited here resembles the phenotype of humans with MG and MuSK antibodies (Fig. 7). In the intricate and convoluted synaptic folds, the high density of voltage gated sodium channels in the membranes' depths amplify the end-plate current, thus enhancing neuromuscular transmission and muscle contraction.[30] A reduction in the size and branching of the motor terminals contributes to the reduced ACh output, and reduced post-synaptic folding increases the threshold for generation of muscle fiber action potential. These structural abnormalities in NMJ, including both pre- and post-synaptic structures, thus impair neuromuscular transmission in rabbits with EAMG.[28]

Intriguingly, similar abnormalities of NMJ structure were also observed in rats with reduced expression of MuSK as noted by RNA interference,[22] in a patient with congenital myasthenic syndromes (CMS) caused by MuSK mutations and also in mice expressing the MuSK missense mutation seen by electroporation experiments.[31] MuSK knockout mice also displayed presynaptic defects in addition to postsynaptic defects, indicating that MuSK is required for retrograde signals, so far unidentified, to maintain the presynaptic structure in mature NMJ.

Dok-7 is required for the maintenance of NMJ

In 2006, a MuSK-interacting protein called Dok-7 was discovered[32] and identified as a member of the Dok family of cytoplasmic proteins. Dok-7 is postulated to have three main functional domains: (i) a pleckstrin homology (PH) domain, essential for membrane association; (ii) a phosphotyrosine-binding (PTB) domain involved in the Dok-7 induced activation of MuSK; and (iii) a large C-terminal domain containing multiple tyrosine residues. Dok-7 knockout mice showed a marked disruption of neuromuscular synaptogenesis that was indistinguishable from the features found in MuSK-deficient mice. Thus, Dok-7 is essential for neuromuscular synaptogenesis through its interaction with MuSK.

Mutations in the Dok-7 protein cause the genetic form of limb-girdle myasthenia called CMS.[33] Some clinical features of these patients resemble those in the severe type of MG accompanied by MuSK antibodies.[34] Proximal muscles are usually more affected than those in distal regions, as evident in MuSK MG patients, and ptosis is often present. However, limb-muscle weakness is comparatively less severe. Previous studies showed no reduction of AChR clustering with significant changes in NMJ of MuSK MG patients,[26] but further structural analysis of NMJ is required in muscles where severe weakness occurs commonly. The weakness and atrophy are not observed uniformly in muscles of these patients, although both MuSK and Dok-7 are essential for the formation of NMJ during the embryonic stage.[32] Notably, one of the major distinctions between acquired MuSK MG and CMS with the Dok-7 mutation is the timing when weakness begins. The CMS patients typically have difficulty in walking after reaching that normal motor milestone during early childhood, whereas the onset of weakness of MG patients, in most instances, occurs in adulthood. Interestingly, AChR clustering and post-synaptic folds are reduced and have small motor terminals as observed at NMJ in patients with CMS and Dok-7 mutations.[35] The effect of Dok-7 mutations on post-synaptic structures might also be an alteration of retrograde signaling to the pre-synaptic nerve terminals resulting in a reduced NMJ size in these patients (Fig. 7). Dok-7, along with MuSK, is also required for the maintenance of NMJ, not only for synaptogenesis.

MuSK plays important roles in the maintenance of NMJ

We have shown that MuSK is required for the maintenance as well as the generation of NMJ.[23,28] Disruption of those mechanisms by MuSK antibodies causes MG in humans. Use of an experimental model for MG showed that MuSK antibodies mediate the pathogenesis of this syndrome in rabbits and mice.[23,27,28] In most cases, the symptoms take more than 3 months to manifest themselves in rabbits and more than 4 weeks in mice. Furthermore, the symptoms were also induced

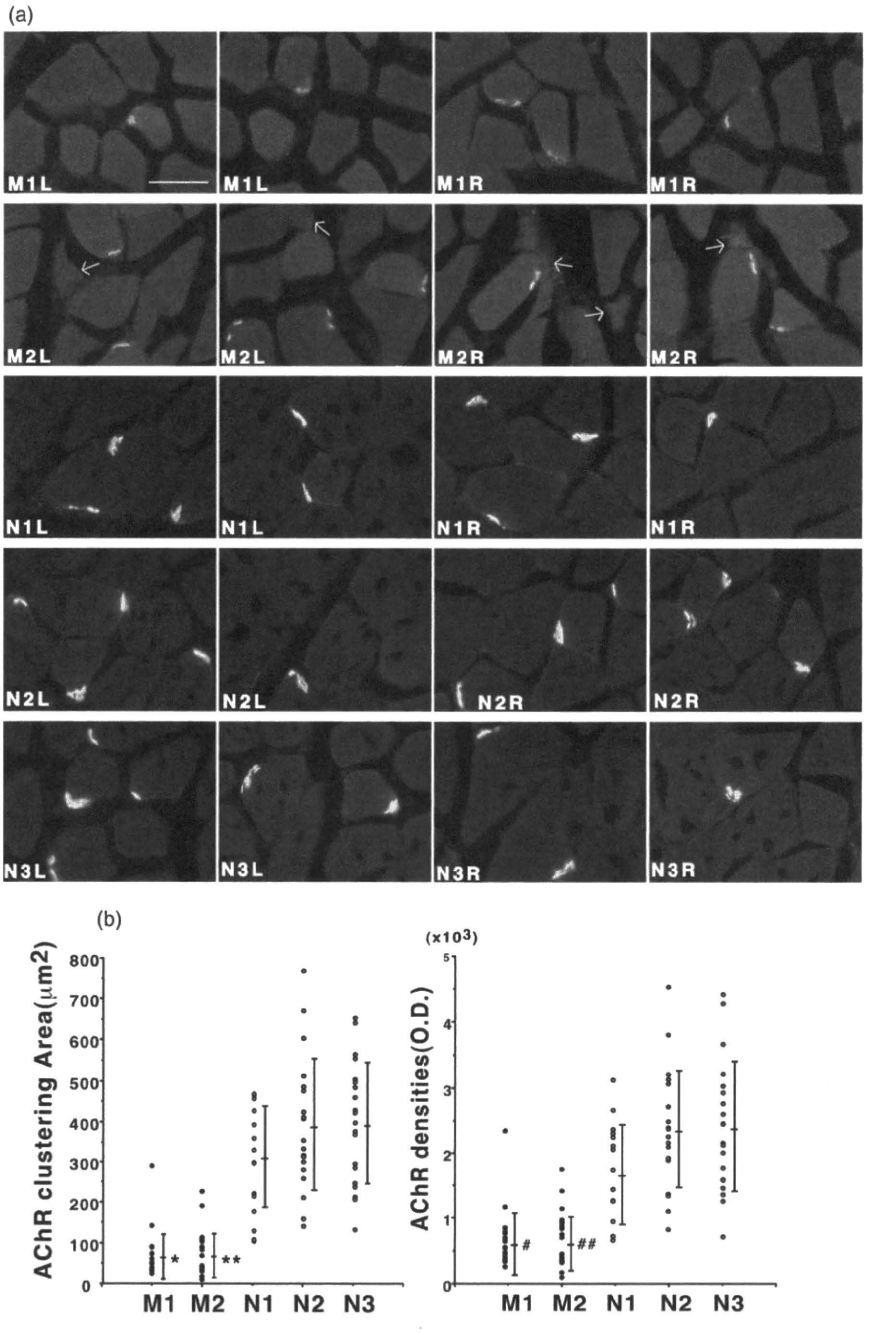

Figure 6 Reduction of the size and density of acetylcholine receptors (AChR) clusters at the neuromuscular junctions (NMJ) in paretic rabbits. (a) Cross-sections from the soleus muscles of two paretic (M1 and M2) and three normal rabbits (N1, N2, and N3) were stained with 10 nM rhodamine-conjugated BTX. Bright crescents of bound BTX, indicative of endplate AChR, were smaller and less intense in the paretic rabbits' muscle fibers than in those of the normal rabbits. Arrows indicate the small angular fibers in M2 soleus muscles (L, left; R, right; bar, 50 μm). (b) Images of ten AChR clusters at NMJ in the right and ten in the left soleus muscles of the paretic and normal rabbits were randomly recorded by a digital imaging camera. Quantification of the area and intensity of AChR clustering in the unprocessed images were measured using NIH Image software. Bars indicate means ± SD. $^{*,**,\#,\#\#}P < 0.01$ versus normal rabbits. *J. Clin. Invest.* 2006; **116**: 1016–1024. Copyright 2009 The American Society for Clinical Investigation.

experimentally by passive transfer of MuSK antibodies from MG patients into animal hosts. However, the injection of a large amount of human MuSK antibodies into mice can barely induce EAMG.[36] The mechanisms used by these antibodies include multiple events during which MuSK functions stall in their process of regulating synapse formation and maintenance.[37] MuSK antibodies against compound antigenic

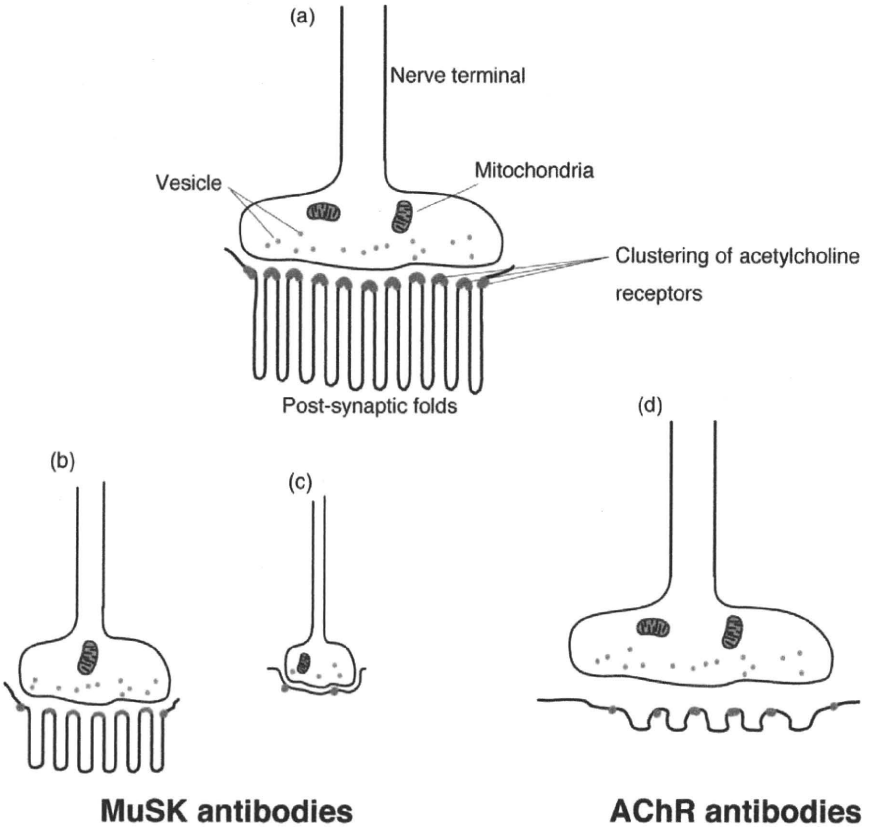

Figure 7 Schematic appearance of neuromuscular junctions (NMJ) observed in normal humans and myasthenia gravis (MG) patients. (a) Normal NMJ. Acetylcholine receptors (AChR) are concentrated at the peaks of abundant and well-preserved, highly complex convoluted junctional folds. (b,c) NMJ observed in experimental animals that model MG was induced by muscle-specific kinase (MuSK) antibodies and in patients with the congenital myasthenic syndrome from MuSK or Dok-7 mutations. Small NMJ in both pre- and post-synaptic structures. (b) Attenuation of AChR and reduced complexity of synaptic folds at post-synaptic membranes without widened synaptic spaces. (c) Disappearance of post-synaptic folds with preserved synaptic space. (d) NMJ in MG patients with AChR antibodies. The myasthenic junction has a reduced number of AChR, simplified synaptic folds and a widened synaptic space with a normal nerve terminal.

determinants in the extracellular domain might engage in their pathogenic activities through antigenic modulation and/or restraint of MuSK functions, and the consequences of these effects range from a partial to entire loss of MuSK function without the involvement of complement-mediated damage. The point that MuSK antibodies in MG patients are mainly of the IgG4 subclass, which does not activate complement, might be relevant here. These diverse possibilities reflect the complexity of clinical features seen in such patients ranging from typical MG throughout its many variants.

Aging and NMJ

How can we extend the studies of MG to understand sarcopenia? The structural changes of NMJ in aged rats have suggested that active remodeling mechanisms at the synapse between nerve and muscle might play crucial roles in the progression of sarcopenia (Fig. 8).[38] Our studies of MG with MuSK antibodies showed that the structure of NMJ is not statically maintained; rather, the nerve-to muscle and muscle-to-nerve signals stimulate dynamic assembly and disassembly of NMJ's molecular complexes. A steady flow of molecular complexes at NMJ sustains both the structures and functions of the motor system, including motoneurons and muscles. However, we do not completely understand the molecular mechanisms, although our animal models of MG showed clearly that muscle-to-nerve signal transduction requires the maintenance of NMJ.[37] We think that studying MG caused by the failure of NMJ maintenance will facilitate further progress in resolving the molecular basis of muscle atrophy. Additional areas of relevance are the many physical conditions, including aging, injury, cancer or AIDS, in which muscles shrink or atrophy. Understanding the molecular basis of NMJ maintenance promises to provide new targets for innovative therapeutics to create healthy, enduring muscles.

Acknowledgments

We thank Ms P. Minick for excellent editorial assistance. This study was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan; by a grant from the Health Science Research Grants for Research on Psychiatric and Neurological Diseases and Mental Health from the Ministry of Health, Labor and Welfare, Japan and by a grant from the Kato Memorial Trust for Nambyo Research.

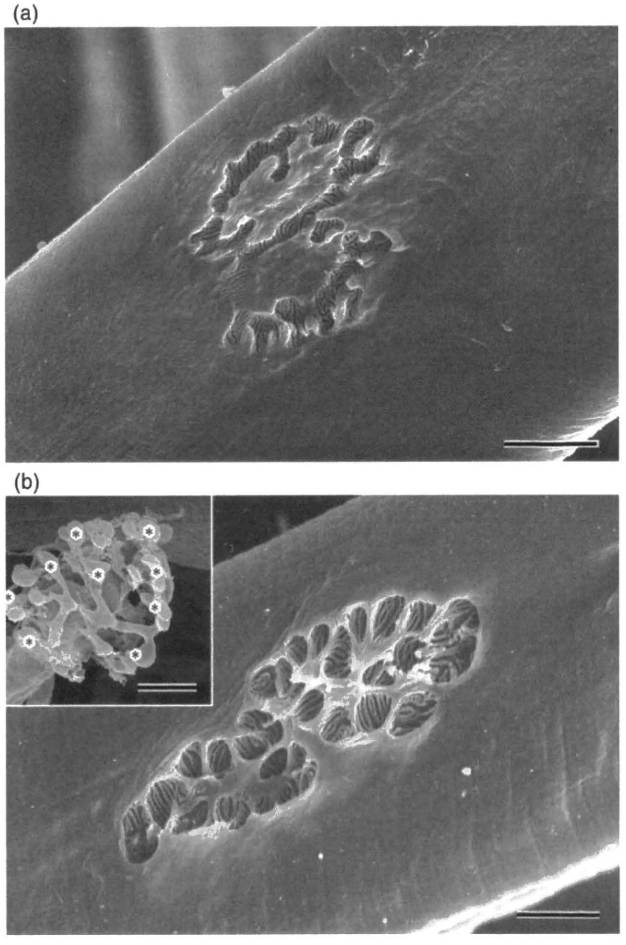

Figure 8 Scanning electron micrographs of neuromuscular junctions (NMJ) in extensor digitorium longus muscles of young and aged subjects. (a) In a 4-month-old rat, convoluted and winding synaptic gutters with numerous slit-like junctional folds. (b) In a 22-month-old rat, a number of cup-like depressions with slit-like junctional folds link together. A nerve ending, which faces toward the muscle apparatus, consists of numerous small protrusions of the terminal axons that might represent individual depressions (magnification, ×3000; bar, 5 μm). Reprinted from Desaki *et al. Virchows Arch.* 2000; **437**: 388–395. Copyright 2009 with permission from Springer.

Conflicts of interest

None.

References

1 Conti-Fine BM, Milani M, Kaminski HJ. Myasthenia gravis: past, present, and future. *J Clin Invest* 2006; **116**: 2843–2854.
2 Vincent A, Lang B, Kleopa KA. Autoimmune channelopathies and related neurological disorders. *Neuron* 2006; **52**: 123–138.
3 Patrick J, Lindstrom J. Autoimmune response to acetylcholine receptor. *Science* 1973; **180**: 871–872.
4 Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med* 2001; **7**: 365–368.
5 Fambrough DM, Drachman DB, Satyamurti S. Neuromuscular junction in myasthenia gravis: decreased acetylcholine receptors. *Science* 1973; **182**: 293–295.
6 Engel AG, Sahashi K, Lamber EH, Howard FM. *The Ulstrastructureal Localization of the Acetylcholine Receptor, Immunoglobulin G and the Third and Ninth Complement Components at the Moter End-Plate and Their Implications for the Pathogenesis of Myasthenia Gravis*. Amsterdam: Excerpta Medica, 1979.
7 Katirji B. *Electrodiagnosis of Neuromuscular Juncion Disorders*. New Jersey: Humana Press Inc., 2003.
8 Vincent A, Bowen J, Newsom-Davis J, McConville J. Seronegative generalised myasthenia gravis: clinical features, antibodies, and their targets. *Lancet Neurol* 2003; **2**: 99–106.
9 Evoli A, Tonali PA, Padua L *et al.* Clinical correlates with anti-MuSK antibodies in generalized seronegative myasthenia gravis. *Brain* 2003; **126**: 2304–2311.
10 Sanders DB, El-Salem K, Massey JM, McConville J, Vincent A. Clinical aspects of MuSK antibody positive seronegative MG. *Neurology* 2003; **60**: 1978–1980.
11 Yeh JH, Chen WH, Chiu HC, Vincent A. Low frequency of MuSK antibody in generalized seronegative myasthenia gravis among Chinese. *Neurology* 2004; **62**: 2131–2132.
12 Ohta K, Shigemoto K, Kubo S *et al.* MuSK antibodies in AChR Ab-seropositive MG vs AChR Ab-seronegative MG. *Neurology* 2004; **62**: 2132–2133.
13 Ohta K, Shigemoto K, Kubo S *et al.* MuSK Ab described in seropositive MG sera found to be Ab to alkaline phosphatase. *Neurology* 2005; **65**: 1988.
14 Ohta K, Shigemoto K, Fujinami A, Maruyama N, Konishi T, Ohta M. Clinical and experimental features of MuSK antibody positive MG in Japan. *Eur J Neurol* 2007; **14**: 1029–1034.
15 Wolfe GI, Oh SJ. Clinical phenotype of muscle-specific tyrosine kinase-antibody-positive myasthenia gravis. *Ann N Y Acad Sci* 2008; **1132**: 71–75.
16 Evoli A, Bianchi MR, Riso R *et al.* Response to therapy in myasthenia gravis with anti-MuSK antibodies. *Ann N Y Acad Sci* 2008; **1132**: 76–83.
17 Strochlic L, Cartaud A, Cartaud J. The synaptic muscle-specific kinase (MuSK) complex: new partners, new functions. *Bioessays* 2005; **27**: 1129–1135.
18 Kummer TT, Misgeld T, Sanes JR. Assembly of the postsynaptic membrane at the neuromuscular junction: paradigm lost. *Curr Opin Neurobiol* 2006; **16**: 74–82.
19 Glass DJ, Bowen DC, Stitt TN *et al.* Agrin acts via a MuSK receptor complex. *Cell* 1996; **85**: 513–523.
20 Kim N, Stiegler AL, Cameron TO *et al.* Lrp4 is a receptor for Agrin and forms a complex with MuSK. *Cell* 2008; **135**: 334–342.
21 Zhang B, Luo S, Wang Q, Suzuki T, Xiong WC, Mei L. LRP4 serves as a coreceptor of agrin. *Neuron* 2008; **60**: 285–297.
22 Kong XC, Barzaghi P, Ruegg MA. Inhibition of synapse assembly in mammalian muscle in vivo by RNA interference. *EMBO Rep* 2004; **5**: 183–188.
23 Shigemoto K, Kubo S, Maruyama N *et al.* Induction of myasthenia by immunization against muscle-specific kinase. *J Clin Invest* 2006; **116**: 1016–1024.

24 Akaaboune M, Grady RM, Turney S, Sanes JR, Lichtman JW. Neurotransmitter receptor dynamics studied in vivo by reversible photo-unbinding of fluorescent ligands. *Neuron* 2002; **34**: 865–876.
25 Lindstrom J. Is "seronegative" MG explained by autoantibodies to MuSK? *Neurology* 2004; **62**: 1920–1921.
26 Shiraishi H, Motomura M, Yoshimura T *et al.* Acetylcholine receptors loss and postsynaptic damage in MuSK antibody-positive myasthenia gravis. *Ann Neurol* 2005; **57**: 289–293.
27 Jha S, Xu K, Maruta T *et al.* Myasthenia gravis induced in mice by immunization with the recombinant extracellular domain of rat muscle-specific kinase (MuSK). *J Neuroimmunol* 2006; **175**: 107–117.
28 Shigemoto K, Sachiho K, Chen J *et al.* Experimentally induced myasthenia gravis with muscle-specific kinase. *Ann N Y Acad Sci* 2008; **1132**: 93–98.
29 DeChiara TM, Bowen DC, Valenzuela DM *et al.* The receptor tyrosine kinase MuSK is required for neuromuscular junction formation in vivo. *Cell* 1996; **85**: 501–512.
30 Wood SJ, Slater CR. Safety factor at the neuromuscular junction. *Prog Neurobiol* 2001; **64**: 393–429.
31 Chevessier F, Faraut B, Ravel-Chapuis A *et al.* MUSK, a new target for mutations causing congenital myasthenic syndrome. *Hum Mol Genet* 2004; **13**: 3229–3240.
32 Okada K, Inoue A, Okada M *et al.* The muscle protein Dok-7 is essential for neuromuscular synaptogenesis. *Science* 2006; **312**: 1802–1805.
33 Beeson D, Higuchi O, Palace J *et al.* Dok-7 mutations underlie a neuromuscular junction synaptopathy. *Science* 2006; **313**: 1975–1978.
34 Palace J, Lashley D, Newsom-Davis J *et al.* Clinical features of the DOK7 neuromuscular junction synaptopathy. *Brain* 2007; **130**: 1507–1515.
35 Slater CR, Fawcett PR, Walls TJ *et al.* Pre- and post-synaptic abnormalities associated with impaired neuromuscular transmission in a group of patients with "limb-girdle myasthenia". *Brain* 2006; **129**: 2061–2076.
36 Cole RN, Reddel SW, Gervasio OL, Phillips WD. Anti-MuSK patient antibodies disrupt the mouse neuromuscular junction. *Ann Neurol* 2008; **63**: 782–789.
37 Shigemoto K. Myasthenia gravis induced by autoantibodies against MuSK. *Acta Myol* 2007; **26**: 185–191.
38 Ezaki T, Oki S, Matsuda Y, Desaki J. Age changes of neuromuscular junctions in the extensor digitorum longus muscle of spontaneous thymoma BUF/Mna rats. A scanning and transmission electron microscopic study. *Virchows Arch* 2000; **437**: 388–395.

第52回日本老年医学会学術集会記録
(若手企画シンポジウム2:サルコペニアの臨床)

1. シナプスを介したサルコペニアの発症メカニズムとバイオマーカー

重本 和宏

要 約 サルコペニア(加齢性筋肉減少症)は高齢者のADL(activity of daily living)とQOL(quality of life)を損なう主要な原因である.サルコペニアの早期発見,運動機能障害者に対するリハビリの効果の判定を可能にする,客観的かつ有効なバイオマーカーが介護予防対策に必要である.加齢による筋の老化促進の要因は,体内環境全体の変化,幹細胞(サテライト細胞)の老化,筋と運動神経細胞の相互作用維持システムの老化の三種類に分類することができる.それらのメカニズムに関する新しい知見をもとに,サルコペニアに対して真に有効なバイオマーカーが開発されるかもしれない.

Key words:サルコペニア,バイオマーカー,炎症性サイトカイン,神経筋シナプス

(日老医誌 2011:48:42-43)

サルコペニアの特徴は加齢による筋肉量低下と筋力低下だが,実際に臨床や介護現場で有効に役立てることができる定義と診断基準のいずれについても確立されていない.サルコペニアは遺伝因子と環境要因に加えて,エピジェネティックの変化など多様な老化促進因子が長時間重なっておきるため,その病態とメカニズムを解明することが困難である.サルコペニアは多様な原因により筋萎縮へ収束する病態の集合群であり,まだ単一の概念として捉えているだけかもしれない.明らかな原因疾患(悪性腫瘍,重篤な感染症,脳血管障害,認知症)による筋肉喪失(cachexia)とサルコペニアの違いの明確な定義はないが,cachexiaのケースで基礎疾患が改善された後のリハビリによる回復力の違いは,サルコペニアの成因と関連する可能性がある.

サルコペニアの診断には画像診断とバイオマーカーが有望である.CTやMRIなどの画像診断は被検者の負担は少ないが,例えば加齢による筋の質的変化を早期の段階から検出する方法はまだ確立されていない.バイオマーカーは検診や疫学調査など被検数が多くてもスクリーニング調査が可能であるため,二重エネルギーX線吸収法(dual energy X-ray absorption)やCTによる筋量測定,運動機能テストと組み合わせて種々のバイオマーカーを使った疫学調査がまだ多くはないがこれまでも報告されている.バイオマーカーの変動とサルコペニアとの直接の因果関係は必ずしも明確でなくとも,統計的に相関が有意であれば疫学研究に利用することができる.しかし,実際に臨床や介護現場で有用な指標となるためには,早期発見と予防に対する有効性を検討する必要がある.今後,サルコペニアのメカニズムに関する知見が深まれば,早期発見やリハビリの効果判定に対して真に有効な画期的なバイオマーカーが開発されるかもしれない[1].

加齢による筋の老化促進の要因は以下の三種類に分類できると考える(図1).体内環境全体の変化(免疫・炎症,ホルモン,代謝・栄養状態),そして幹細胞(サテライト細胞)とそれを維持する微少環境(ニッチ)の老化,さらに筋と運動神経細胞(中枢神経)の相互作用による維持システムの老化である.これらの原因がお互いに影響しあうことでサルコペニアが進行すると考えている.加齢に伴い変化する体内環境の一つとして,老化現象として慢性炎症状態が顕在化する可能性が提案されている[2].最近では,筋からも炎症性サイトカインを分泌することが明らかにされている[3].炎症性サイトカインは脂肪細胞からも分泌されるが,サルコペニアでは筋萎縮にともない筋肉内の脂肪組織も増大することが指摘されている(sarcopenic obesity).血中のIL-6,TNF-αがサルコペニアによる筋量および筋力低下と相関があるという報告がある[4][5].

我々は,運動神経線維と筋のつなぎ目である神経筋シナプスを介した筋と運動神経の相互作用システムとサル

Biomarkers for sarcopena

Kazuhiro Shigemoto:東京都健康長寿医療センター研究所(東京都老人総合研究所)老年病研究チーム

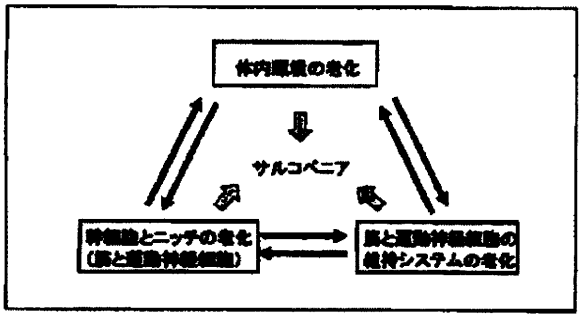

図1　サルコペニアのメカニズム
環境要因，遺伝因子，エピジェネティックの変化で誘発される3種類の経路

コペニアとの関連に注目して研究をおこなっている．もともと健常筋には萎縮へと向かうカスケードが常在している．若い健常人であっても骨折などで筋活動が停止すると，2週間以内で急速に筋萎縮に至る．適切な運動習慣により，運動神経線維と筋のつなぎ目である神経筋シナプスを介した筋と運動神経の相互作用システムが，萎縮カスケードに拮抗することで筋と運動神経の両方が保持されている[6,7]．老化に伴う何らかの原因により，運動神経と筋の相互作用維持メカニズムが阻害されると筋萎縮が誘導されると考えている．このメカニズムを解明することで，サルコペニアの原因解明，診断と予防法の開発ができるかもしれない．

まとめ

筋萎縮に至る前段階で運動能力の可塑性（回復力）を科学的な根拠に基づく方法（バイオマーカー）で定量化することができれば，介護現場においても様々な場面で有効に活用できるであろう．また，サルコペニアを早期に発見することができれば，進行を抑制するための適切な運動処方の開発とその有効性を検討することが可能となる．

文　献

1) Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al: Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol 1998; 147: 755–763.
2) Handschin C, Spiegelman BM: The role of exercise and PGC1alpha in inflammation and chronic disease. Nature 2008; 454: 463–469.
3) Wenz T, Rossi SG, Rotundo RL, Spiegelman BM, Moraes CT: Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging. Proc Natl Acad Sci U S A 2009; 106: 20405–20410.
4) Visser M, Pahor M, Taaffe DR, Goodpaster BH, Simonsick EM, Newman AB, et al: Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. J Gerontol A Biol Sci Med Sci 2002; 57: M326–332.
5) Schaap LA, Pluijm SM, Deeg DJ, Harris TB, Kritchevsky SB, Newman AB, et al: Higher inflammatory marker levels in older persons:associations with 5-year change in muscle mass and muscle strength. J Gerontol A Biol Sci Med Sci 2009; 64: 1183–1189.
6) Shigemoto K, Kubo S, Mori S, Yamada S, Miyazaki T, Akiyoshi T, et al: The Immunopathogenesis of Experimetal Autoimmune Myasthenia Gravis induced by Autoantibodies Against Muscle-specific kinase (MuSK EAMG). In: Myasthenia Gravis Disease Mechanisms and Immune Intervention, Christadoss P (ed), Linus Publications, Deer Park, NY 11729, 2009, p305–326.
7) 重本和宏：サルコペニア診断のためのバイオマーカー．サルコペニアの基礎と臨床，真興交易医書出版部，東京，2010（印刷中）．

地域在住高齢者におけるサルコペニア改善のための運動，アミノ酸補充の効果

[1]東京都健康康長寿医療センター研究所，[2]味の素株式会社，
[3]国立長寿医療センター研究所
金　憲経[1]，齋藤京子[1]，吉田英世[1]，加藤弘之[2]，小林久峰[2]，片山美和[2]，鈴木隆雄[3]

Effects of exercise and amino acid supplementation for sarcopenia in community-dwelling elderly people

Hun-kyung Kim[1], Kyoko Saito[1], Hideyo Yoshida[1], Hiroyuki Kobayashi[2], Hisamine Kato[2], Miwa Katayama[2] and Takao Suzuki[3]

[1]Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
[2]Ajinomoto Co. Inc., Kawasaki, Japan
[3]National Institute for Longevity Sciences, Aichi, Japan

アミノ酸研究　**4**(1)，55－57，2010

アミノ酸研究 Vol. 4 , No. 1 , (2010)

地域在住高齢者におけるサルコペニア改善のための運動，アミノ酸補充の効果

[1]東京都健康康長寿医療センター研究所，[2]味の素株式会社，
[3]国立長寿医療センター研究所
金 憲経[1]，齋藤京子[1]，吉田英世[1]，加藤弘之[2]，小林久峰[2]，片山美和[2]，鈴木隆雄[3]

Effects of exercise and amino acid supplementation for sarcopenia in community-dwelling elderly people

Hun-kyung Kim[1], Kyoko Saito[1], Hideyo Yoshida[1], Hiroyuki Kobayashi[2], Hisamine Kato[2], Miwa Katayama[2] and Takao Suzuki[3]

[1]Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
[2]Ajinomoto Co. Inc., Kawasaki, Japan
[3]National Institute for Longevity Sciences, Aichi, Japan

はじめに

人間の諸機能は，常に変化する属性を持ち，個体の潜在能力が効率よく発揮できる方向へ変えていくのが一般的である。しかし，中年期を過ぎると様々な組織の機能が十分発揮できなくなり，環境変化への適応能力の低下ないしは機能喪失が徐々に増してくる。その背景要因の一つに，体脂肪やLBM（lean body mass）の変化が挙げられる[1)]。中でも，骨格筋量の減少（Sarcopenia）[2)]は，筋力の衰え，身体機能の低下をもたらし，身体的障害あるいは老年症候群の発症と密接に関わっていることが多くの疫学調査で指摘されている。骨格筋量の減少には性，年齢，身長，体重，BMI，テストステロン，脂肪量，不活動，ビタミンD，低栄養など様々な要因が複雑に関わっている[3,4)]。サルコペニア予防策を構築するためには，多くの危険因子の中で，可変因子の改善を目的とした取組みが有効であり，Fiataroneら（1994）は，骨格筋の不使用と低栄養の改善に焦点を当てた介入が有効であると指摘している[5)]。

サルコペニア予防のための戦略

加齢に伴う骨格筋量の減少を予防したり，委縮した骨格筋の機能を回復させるためには，筋に適当な刺激を与えるトレーニングが有効的と考える。しかし，虚弱高齢者を対象とする場合には，筋力発揮に伴うメカニカルストレスの増大や循環器への負担が懸念され，無理のないトレーニングが原則である。運動効果について調べた研究によれば，日常的活動レベルが低く，筋力低下が進んでしまった虚弱高齢者であっても筋力増大の効果が報告されている。虚弱高齢者における著しい筋力増大効果は，筋肥大よりも神経系の機能改善に起因するものと考えられてきた。しかし，最近の研究により高齢者でも筋肥大が起こることが確かめられている[5)]。

Fiataroneら（1994）は，72～98歳の長期施設入所者100名を対象に，筋力強化運動，栄養補充効果を検証した。その結果，筋力強化運動群では筋力113%（P<0.01），歩行速度11.8%（P=0.02），階段昇降機能28.4%（P=0.01）と有意に上昇したが，太腿の筋断面積2.7%（P=0.11）増加に止まった。一方，240mlの栄養補充（炭水化物60%，脂肪23%，タンパク質17%）の効果は検証されなかったと指摘している[6)]。これらの結果は，サルコペニアの改善のためには単なる栄養補充ではなくて，骨格筋量の減少メカニズムを把握した上での処置が必要であることを示唆する試験である。高齢者における骨格筋量の減少（サルコペニア）背景は，高齢者では，筋タンパク質の合成と分解が減弱し，その結果としてサルコペニアが起こるということである。よって，骨格筋量の予防・改善には筋タンパク質合成促進が有効と考える。骨格筋タンパク質合成は血液中のアミノ酸濃度に影響され，血液中のアミノ酸濃度が上昇すると筋タンパク質合成速度が速やかに増加するが，分解速度は変化しないことが指摘されている[7)]。特に，高ロイシン含量の必須アミノ酸は比較的少量で筋タンパク質合成が促進されることを検証したことから，その長期摂取による骨格筋量の改善が期待できる[8)]。

サルコペニア改善のための運動，アミノ酸補充の効果

1）サルコペニア高齢者の特徴

これらの背景を踏まえて，筆者は，サルコペニアと判定された304名と正常者1,095名の調査項目を比較し，サルコペニア高齢者の特徴を調べた。その結果，サルコペニア群は正常群に比べて，年齢が高く，下腿三頭筋周囲，BMI，筋肉量が有意に低値を示すとともに，健康度自己

表１．サルコペニア群と正常群の調査項目の比較

項　目	サルコペニア群	正常群	p値
年齢（歳）	79.49 ± 2.93	78.51 ± 2.77	<0.001
下腿三頭筋周囲（cm）	30.17 ± 2.03	33.92 ± 2.60	<0.001
ＢＭＩ（kg/m^2）	18.98 ± 2.01	23.74 ± 2.84	<0.001
筋肉量（kg）	26.92 ± 2.61	31.73 ± 3.16	<0.001
健康度自己評価，健康（%）	75.7	85.8	<0.001
外出頻度，少ない（%）	4.6	2.5	0.051
運動習慣，有（%）	27.3	33.5	0.039
既往歴，有（%）			
高血圧	51.0	58.0	0.029
高脂血症	32.2	40.5	0.009
貧血症	4.6	2.2	0.022
骨粗鬆症	38.2	30.7	0.014
骨折	28.6	22.9	0.038

評価，定期的な運動習慣を持っている者の割合は低かったが，外出頻度低下者の割合は高かった。一方，既往歴においては，貧血症，骨粗鬆症，骨折歴は有意に高かったが，高血圧症，高脂血症は正常群より低かった（表１）。

２）運動，アミノ酸補充の効果

サルコペニア改善のための運動，アミノ酸補充の効果を検証するために，介入参加希望者をRCTにより運動群と栄養群に分け，運動群には週２回，１回当たり60分間の筋力強化と歩行機能の改善を目的とした包括的運動指導を，栄養群にはロイシン高配合のアミノ酸３gを１日２回補充する指導を，３ヶ月間実施した。介入前後における身体組成，体力，老年症候群の改善の度合いを検討した。その結果，LBMは運動群で2.4%，栄養群で4.6%の有意な向上が，歩行速度は，運動群で18.6%，栄養群で10.3%の顕著な向上が確認され（図１），地域在住サルコペニアの改善には運動のみならずアミノ酸補充も有効であることが示唆された。しかし，サルコペニア高齢者に多く観察される尿失禁は，運動群で38.9%から

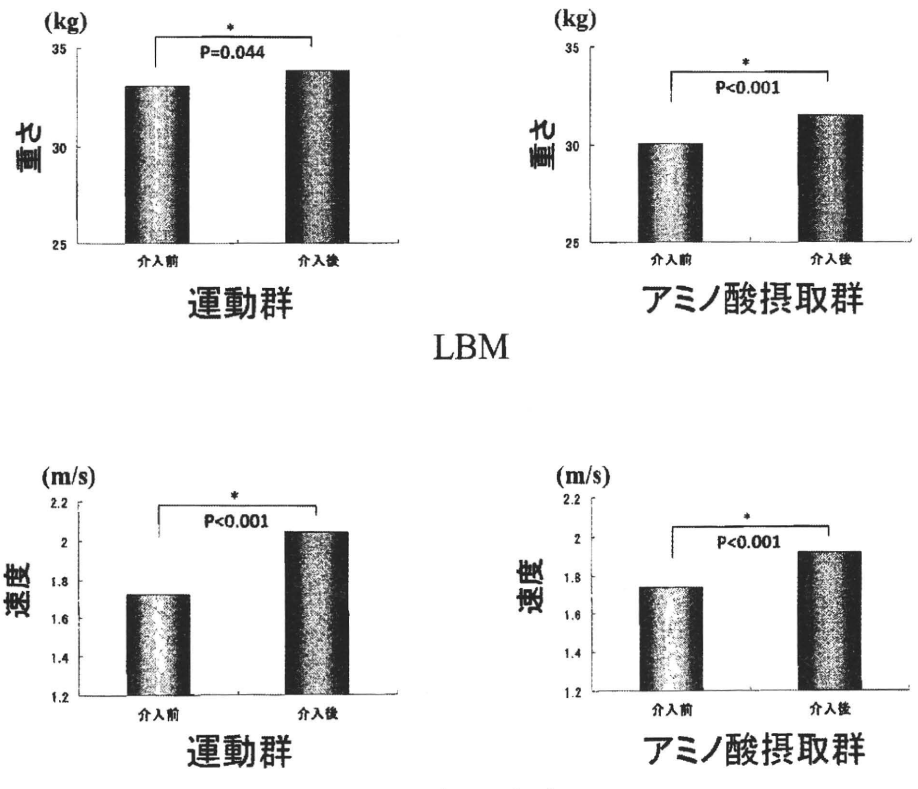

図１．３ヶ月間の運動，アミノ酸摂取の介入がLBMおよび歩行速度に及ぼす影響

19.4％（P=0.021）と有意に改善されたが，栄養群では有意な改善が見られなかった。以上のことから，サルコペニア高齢者のLBMあるいは体力の改善を目的とした場合には，運動指導あるいは栄養補充の両方とも有効な手法であることが確認されたが，サルコペニア高齢者に有症率の高い老年症候群の改善のためには，運動介入の効果が優れる可能性が示唆された。

おわりに

骨格筋量の減少に伴う筋力の衰えを意味するサルコペニアは後期高齢者において有症率が上昇し，身体機能の障害や死亡と強く関連していることが指摘されている。サルコペニアと関連する要因は様々で複雑であるが，不活動や栄養など可変要因の改善に焦点を当てた予防策の効果を検討したところ，骨格筋量の増加，体力の向上には，運動指導，栄養指導ともに有効であった。しかし，サルコペニア高齢者に多く見られる老年症候群の解消には，運動指導がより有効であることを検証した。

文献

1）金憲経，田中喜代次，天貝均，鈴木隆雄（1999）身体組成の加齢に伴う変化：DXA法による検討．*体育学研究* **44**：500-509.
2）Rossenberg IH (1989) Summary comments. *Am J Clin Nutr* **50**：1231-1233.
3）Baumgartner RN, Waters DL, Gallagher D, Morley JE, Garry PJ (1999) Predictors of skeletal muscle mass in elderly men and women. *Mech Ageing Dev* **107**：123-136.
4）金憲経，吉田英世（2010）高齢者におけるサルコペニア発生の現状と関連要因．*Geriat Med* **48**：191-195.
5）Frontera WR, Meredith CN, O'reilly KP, Knuttgen HG, Evans WJ (1988) Strength conditioning in older men: Skeletal muscle hypertrophy and improved function. *J Appl Physiol*, **64**：1038-1044.
6）Fiatarone MA, O' Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, Roberts SB, Kehayias JJ, Lipsitz LA, Evans WJ (1994) Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med* **330**：1769-1775.
7）Bohe J, Low A, Wolfe RR, Rennie MJ (2003) Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: A dose-response study. *J Physiol* **552**：315-324.
8）Borsheim E, Bui QT, Tissier S, Kobayashi H, Ferrando AA, Wolfe RR (2008) Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. *Clin Nutr* **27**：189-195.

Progress in Medicine 第12号 別刷

Vol.30 No.12 2010.12

特集：ロコモティブシンドロームと生活習慣病

3．ロコモティブシンドロームの発症メカニズム

4）サルコペニアと ロコモティブシンドローム

Kim Hunkyung
金　憲経

ライフ・サイエンス

Prog.Med.
30：3043～3047, 2010

ロコモティブシンドロームと生活習慣病

3．ロコモティブシンドロームの発症メカニズム
4）サルコペニアとロコモティブシンドローム

Kim Hunkyung
金　憲経*

*東京都健康長寿医療センター研究所

はじめに

人間の諸機能は，中年期を過ぎると低下ないしは喪失が徐々に増してくる．その背景要因の1つに，体組成の変化が挙げられる．加齢に伴う体組成の変化の中で，最も特徴的なのは脂肪組織量の増加と，骨や骨格筋を含んだ徐脂肪組織量（fat-free mass：FFM）の低下である．加齢に伴うFFMの変化は，男性で0.34 kg/yr，女性で0.22 kg/yr減少することが[1]，筋肉量は，男性で0.19 kg/yr，女性で0.11 kg/yr減少するが，50歳代以降では下肢骨格筋量の減少が顕著であることが指摘されている[2]．加齢に伴って筋肉量や骨格筋量が減少すると，筋の質を表す筋力の衰弱をもたらし，特に下肢筋力の衰えは歩行機能を著しく低下させ，ひいては転倒・骨折の原因となるなど，高齢者の移動能力を制限してしまう重大な要因である．

一般的にロコモティブシンドローム（以下，ロコモ）は，運動器の障害のため移動能力の低下を来し要介護状態になっていたり，要介護状態になる危険性の高い状態を指す概念である．身体活動は骨，筋肉，関節，神経などの組織や器官の機能的連合によって産出される結果であり，どれか1つ不具合になっても上手く働かない．

ここでは，ロコモとサルコペニア（sarcopenia）に共通の媒介要因として考えられる筋力の衰えという観点から，ロコモとサルコペニアの関連性や位置づけについて簡単に紹介する．

表1　性・年齢・人種別にみたサルコペニアの有症率

年齢群（歳）	男性 ヒスパニック（n＝221）	男性 白人（n＝205）	女性 ヒスパニック（n＝209）	女性 白人（n＝173）
＜70	16.9	13.5	24.1	23.1
70～74	18.3	19.8	35.1	33.3
75～80	36.4	26.7	35.3	35.9
＞80	57.6	52.6	60.0	43.2

（文献4より引用）

サルコペニアの定義および有症率

加齢に伴って徐々に起こり得る筋肉量の減少や筋力の衰えを表す言葉として「sarcopenia」が1989年以降使用され[3]，老年症候群の発症と深く関わっていることから注目されるようになっている．

現在サルコペニアの操作的定義として広く用いられているものの1つとしては，Baumgartnerらの定義がある．この定義は，二重エネルギーX線吸収法（dual energy x-ray absorptiometry, DXA）から求めた四肢の筋量（appendicular skeletal muscle mass：ASM）を身長（m^2）で除したskeletal muscle mass index（SMI）を指標としたものである．サルコペニアの定義は，18～40歳成人のSMI平均より2SD以下の場合とされている．この定義に基づく有症率は，70歳以下の高齢者で13.5～24.1%の範囲であるが，80歳以上になると43.2～60.0%に上昇する（表1）．さらに，サルコペニアのカットポイントは，SMIが男性で7.26 kh/m^2，5.45 kg/m^2と

表2　サルコペニア選定に用いた骨格筋量のカットポイント

報告者	筋量の測定法	定　義	男　性	女　性
Baumgartner, et al	DEXA	ASM/Ht², 若年成人2SD↓	7.26	5.45
Tanko, et al	DEXA	ASM/Ht², 若年成人2SD↓	*	5.40
Janssen, et al	BI	SMI	8.50	5.75
Chien, et al	BI	SMI, 若年成人2SD↓	8.87	6.42
Sanada, et al	DEXA	ASM/Ht², 若年成人2SD↓	6.87	5.46

ASM(kg) = appendicular skeletal muscle mass estimated by DXA.
SM(kg) = skeletal muscle mass estimated by BI.
SMI = SM/Ht², Ht = height.　（文献4より引用）

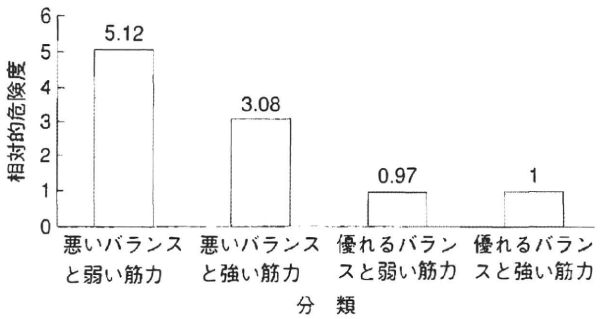

図1　歩行障害の予知因子
（文献5より引用）

提案するとともに，disabilityと密接に関連する（表2）ことから，サルコペニアは高齢期の大きな健康問題としてとらえるべきであると強調している[4]．

歩行機能には筋力とバランスが密接に関わっている

歩行機能は，体力全般の代表的な指標である．外出を楽にし，活動範囲を広げ，元気で長生きを実現するためには，歩行機能の維持・向上は不可欠な要素である．高齢者歩行パターンの特徴は，歩行速度の低下，歩幅の短縮，歩隔の増大，両脚支持時間の延長，遊脚期での足の挙上の低下，腕の振りの減少，不安定な方向転換などである．高齢者に多くみられる歩行機能の低下は，死亡率の上昇，転倒率の増加，生活機能の障害など，様々な指標と密接に関わっていることが多くの研究で指摘されている．

Rantanenらが，65歳以上の高齢女性758名を対象に3年間追跡調査し，歩行障害の発生と関連する要因について検討した結果によれば，「筋力の減少とバランス能力の低下」という条件の対象者は「優れる筋力とバランス機能」を有する対象者に比べて，歩行障害発生の危険性の高いことを指摘し（RR＝5.12，95% CI＝2.68–9.80），歩行機能を維持するためには筋力向上とバランス機能の改善が必要であると強調している（図1）[5]．

サルコペニアの高齢者の特徴

筆者は，大都市部在住の75歳以上の後期高齢女性1,399名を対象に，「四肢の骨格筋量が少ない」「BMIが低い」「膝伸展力が低い」3つの基準に該当する場合をサルコペニアと定義し，該当者304名（21.7%）を抽出し，特徴を調べている．その結果によれば，サルコペニア高齢者は，年齢が高く，下腿三頭筋周囲，BMI，筋肉量は低値を示すとともに健康度自己評価，定期的な運動習慣をもっている者の割合も低いという傾向である．しかし，外出頻度が少ない者の割合は高値を示し，サルコペニアと判定された高齢者は活動量が少なく，自分の健康に対する自信感を喪失している者が多いと推測できる．一方，既往歴においては，貧血症，骨粗鬆症，骨折歴は有意に高い割合を示しているが，高血圧症，脂質異常症は正常群より低い割合を示していることから，サルコペニア高齢者の場合，骨粗鬆症に伴う骨折危険性が高いことが示唆されている（表3）．さらに，サルコペニア高齢者の歩行機能を調べるために，5mの最大歩行速度を計測し，サルコペニア群と正常群を比較したところ，図2に示した通りに，サルコペニア群は1.58±0.34 m/sec，正常群は1.71±0.36 m/secとして，サルコペニア群の歩行速度が有意に低いことが確認されている[6]．

サルコペニアと関連する要因

老化に伴う筋骨格筋量減少の原因としては，加齢，IGF-1の分泌減少，慢性疾患，アンドロゲン・エストロゲン分泌の減少，炎症性サイトカインの増加，身体活