s-IBM DNA-PKcs, we examined a total of 393 vacuolated fibers with 950 nuclei and 1953 myonuclei in non-vacuolated fibers. As it was sometimes difficult to differentiate between the nuclei of invading/surrounding mononuclear cells and myonuclei, we excluded muscle fibers surrounding inflammatory cells from the nucleus calculation. We categorized nuclei as positive when a brown color was clearly discernible against lightly-stained hematoxylin.

3. Results

3.1. Increased expression of the DNA double strand break (DSB) marker γ-H2AX in s-IBM vacuolated fibers

In the non-pathologic controls, a small number of myonuclei showed a weakly positive reaction to γ-H2AX ($n = 5$; $6.0 \pm 1.8\%$, mean ± standard deviation [SD]. Range: 3.97–8.05) (Fig. 1A). In polymyositis and dermatomyositis, the nuclei in regenerating fibers were positive for γ-H2AX. The nuclei in perifascicular atrophic fibers in cases of dermatomyositis were strongly positive (Fig. 1B), and positive myonuclei were also found in other fibers in polymyositis and dermatomyositis. A proportion of the cells in inflammatory exudates were positive for γ-H2AX. In neurogenic muscular atrophy, strongly reactive nuclei were usually found in atrophic angulated fibers (Fig. 1C), and the nuclei at pyknotic nuclear clumps showed increased reactivity for γ-H2AX. In other neuromuscular diseases, the nuclei at nuclear clumps such as those observed in myotonic dystrophy showed increased reactivity for γ-H2AX, and the nuclei in ragged-red fibers in mitochondrial encephalomyopathy and those of regenerating fibers in various myopathies were strongly positive for γ-H2AX. Vacuoles in hypokalemic myopathy, myopathy with autophagic vacuoles, colchicine myopathy, and OPMD were negative for γ-H2AX (Fig. 1D).

In s-IBM, a proportion of fibers contained vacuoles that were partially or entirely lined by positive immunoreactivity (Fig. 1E and F). Table 2 shows the percentage of (1) vacuolated fibers vs. total fibers and (2) fibers containing γ-H2AX positive vacuoles vs. total vacuolated fibers in patients with s-IBM ($n = 10$; $74.0 \pm 13.0\%$, mean ± SD). The nuclei in vacuolated fibers displayed strong γ-H2AX-positive reactivity, and the percentage of positive nuclei was significantly higher in vacuolated fibers than in non-vacuolated fibers (Table 1) ($p < 0.01$; paired Student's t-test). In polymyositis, the percentage of γ-H2AX-positive nuclei ($n = 10$; $23.3 \pm 7.4\%$, mean ± SD) was similar to that in the non-vacuolated fibers in s-IBM, but lower than that in the vacuolated fibers ($p < 0.01$; Student's t-test).

The results of immunoblotting using this anti-γ-H2AX antibody showed several positive bands including ubiquitinated forms of γ-H2AX (Fig. 2) [20].

3.2. Detection of the DSB repair enzyme DNA-PK in s-IBM

In s-IBM, all of the DNA-PK components (DNA-PKcs, Ku70, and Ku80) were found in vacuolar peripheries as well as being strongly expressed in nuclei, consistent with the results for γ-H2AX (Fig. 1G, H and I). As for DNA-PKcs, $70.6 \pm 14.0\%$ (mean ± SD) of vacuolated fibers contained positive vacuoles for DNA-PKcs. The percentage of positive nuclei for DNA-PKcs was significantly higher in vacuolated fibers than in non-vacuolated fibers ($61.7 \pm 10.6\%$, mean ± SD, vs. $32.5 \pm 10.2\%$: $p < 0.01$; paired t-test). Ku70 was often found to form several round or comma-shaped cytoplasmic inclusions in vacuolated fibers and other fibers. We confirmed the relative localization of Ku70, the nuclear envelope, and DNA in a triple-fluorescence study in five patients with s-IBM, five patients with polymyositis, and patients with other diseases. In polymyositis and other controls, Ku70 was confined to within the emerin boundary, even when the Ku70-signal was very intense (Fig. 3A). In vacuolated fibers in s-IBM, although Ku70 was often localized to the nuclei, it was also found in vacuolar peripheries, around the nuclei, and in the cytoplasm (Fig. 3B and C). In a few instances, cytoplasmic Ku70-positive granules were associated with nuclear frag-

Table 2
Quantitation.

s-IBM Pt number	Vacuolated fibers/total fibers (%)	γ-H2AX positive fibers/vacuolated fibers (%)	Positive nuclei in	
			Vacuolated fibers (%)	Non-vacuolated fibers (%)
1	18.6	87.2	64.0	24.5
2	21.0	82.9	64.0	29.0
3	5.0	69.6	64.5	39.4
4	4.2	76.5	70.3	23.8
5	7.2	89.5	81.4	38.2
6	23.0	76.8	65.0	22.1
7	6.6	47.1	58.8	8.5
8	7.6	74.6	56.5	25.2
9	4.2	58.1	58.2	24.9
10	7.6	78.3	63.9	21.6
Mean ± SD	10.5 ± 7.3	74.0 ± 13.0	64.7 ± 7.1*	25.7 ± 8.8

* The percentages for vacuolated fibers are significantly higher than those for non-vacuolated fibers ($p < 0.01$).

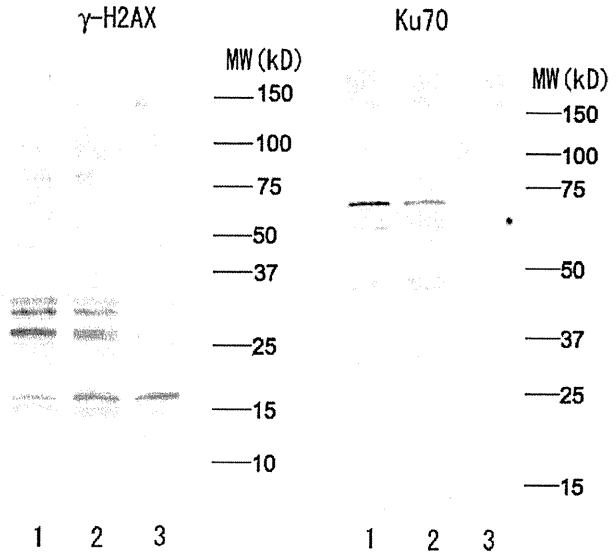

Fig. 2. Tests for the antibody specificity in immunoblotting. Muscle homogenates in control patients were segregated through polyacrylamide gel electrophoresis and immunoblotted using anti-γ-H2AX (left) and Ku70 (right) antibodies. The molecular weights of γ-H2AX and Ku70 are 15 kDa and 70 kDa, respectively. In γ-H2AX, patient 1 and 2, the extra bands between 25 kDa and 37 kDa correspond to the ubiquitinated forms [20]. In Ku70, patient 1 and 2, positive bands appear around its molecular weight.

ments, indicating nuclear breakdown. Ku70-positive deposits were sometimes found around intact nuclei (Fig. 3D).

Immunoblotting of muscle homogenates with the anti-Ku70 antibody showed a clear band around the molecular weight (Fig. 2).

3.3. Localization of HNE, iNOS, and LAMP-2

ROS is an inducer of DSB in muscle cells and oxidative stress may be associated with vacuolar formation [21], so we tested 4-hydroxy-2-noenal (HNE), a product of lipid peroxidation by ROS [22], and iNOS, a marker of oxidative stress that was previously found to be increased in vacuolated fibers [21]. HNE and iNOS were increased not only in some vacuolated fibers in s-IBM, but also in perifascicular atrophic fibers in dermatomyositis and ragged red fibers. In non-vacuolated fibers in s-IBM, atrophic fibers in neurogenic muscular atrophy, and pyknotic nuclear clumps, the two ROS markers were not increased.

Several studies indicated that rimmed vacuoles are lysosomes in origin [23,24]. In this study, we observed that vacuoles in s-IBM usually showed positive for the lysosome marker LAMP-2, as described previously [23]. A dual fluorescence study using antibodies against LAMP-2 and emerin showed frequent association of these two markers in the vacuoles in s-IBM.

3.4. Immuno-electron microscopy of Ku70

In the ultrastructural study of Ku70 in s-IBM, we detected Ku70-positive granules in some nuclei. Ku70-positive granules were often found in the vacuolar spaces of

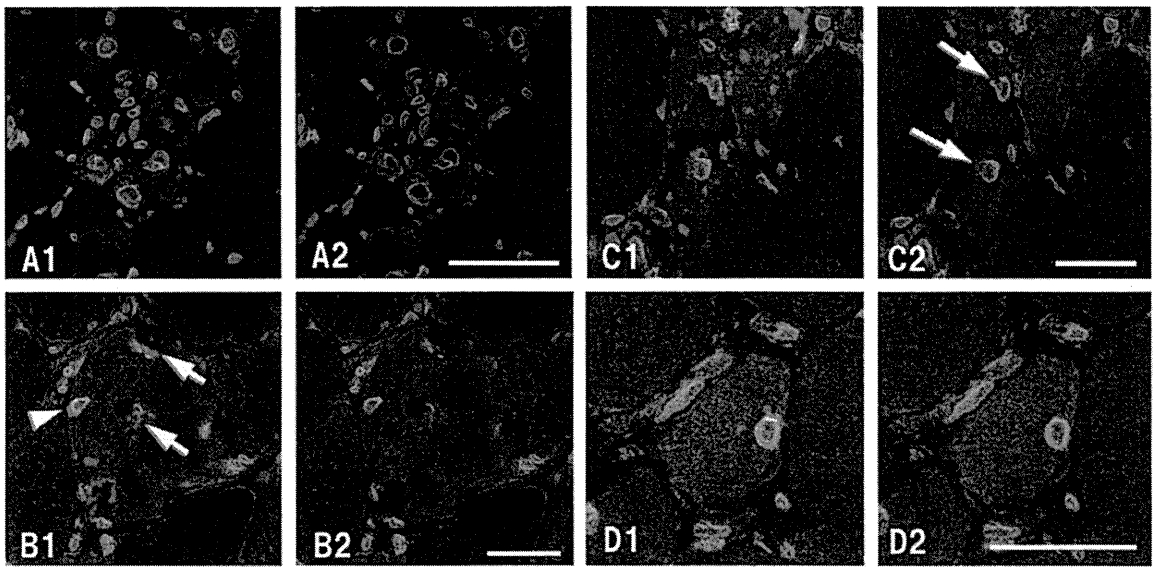

Fig. 3. Triple fluorescence study. Ku70 (red), emerin (green), and DNA (DAPI: blue). A: Regenerating fibers. B to D: s-IBM. A1–D1: overlay of the three colors. A2–D2: emerin plus DNA. (A) Ku70-positive deposits are largely confined to the area surrounded by emerin. (B) A vacuolated fiber contains a nucleus abutted by deposits of Ku70 (arrow head). Fragments of Ku70-positive deposits intermingle with remnants of emerin or DNA (arrows). The figures indicate nuclear breakdown and impaired incorporation of Ku70 into the nucleus. (C) Muscle fibers with numerous cytoplasmic deposits of Ku70 and breaks in the nuclear envelope (arrows). (D) Ku70-positive deposits surrounding a nucleus with an intact circle of emerin. This figure shows that nuclear import of Ku70 is impaired even in the early phase of nuclear breakdown in s-IBM. Bar = 25 μm.

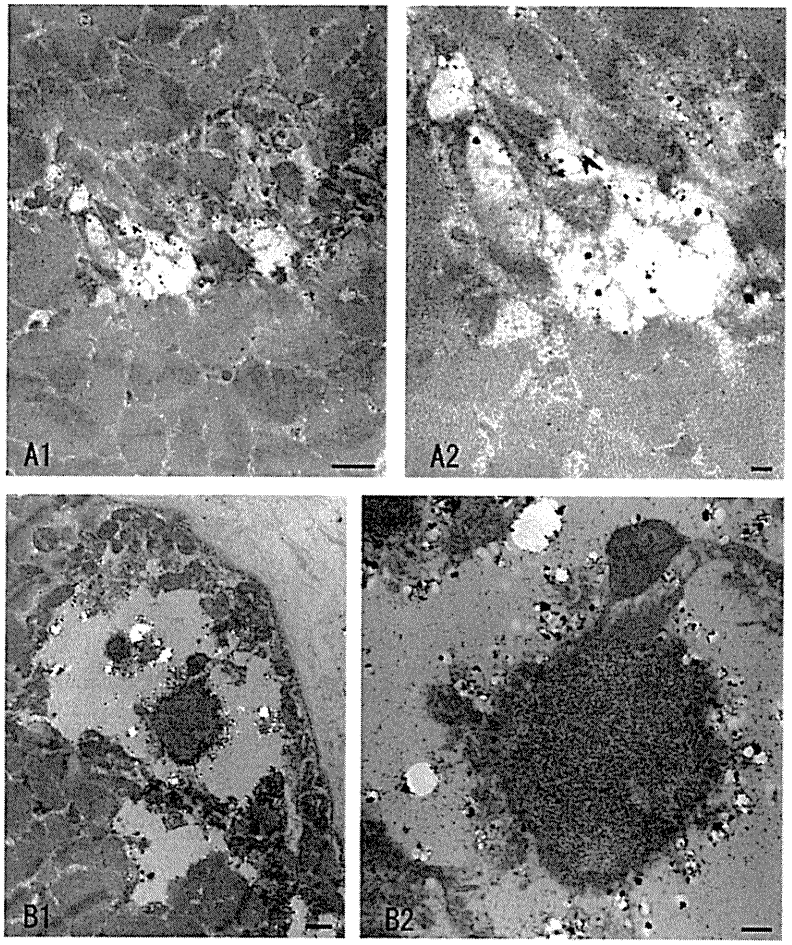

Fig. 4. Immunoelectron microscopy of Ku70, a regulatory component of DNA-PK. (A1) Deposits of Ku70 in cytoplasmic spaces. (A2) Higher magnification of A1. The positive reactivity may correspond to the cytoplasmic inclusion of Ku70 in immunofluorescence. (B1) Ku70 surrounding electron-dense round bodies. (B2) Higher magnification of one body shows granular structures inside with a peripheral dense zone. According to the triple fluorescence results, such as those shown in Fig. 3, the round body corresponds to a degenerating nucleus, and the electron micrograph may illustrate that the nucleus cannot incorporate Ku70. Bar = 1 μm (A1 & B1), 200 nm (A2), 500 nm (B2).

various sizes, sometimes combined with degenerative products (Fig. 4A). Ku70-positive products were also found to be attached to something like degenerating nuclear structures that contained no Ku70 (Fig. 4B), which may have corresponded to nuclei surrounded by Ku70-positive deposits in the immunofluorescence study (Fig. 3B, arrowhead).

4. Discussion

4.1. Findings in s-IBM

In the current study, we showed that the percentage of DSB-positive nuclei was significantly higher in vacuolated fibers than in other fibers in s-IBM. This finding suggests that nuclear breakdown along with the accumulation of DSB occurs in muscle cells in s-IBM. Moreover, we detected figures suggesting impaired nuclear import of Ku70. Nuclear translocation of Ku proteins is important for DBS repair, and a deficiency in nuclear translocation caused hypersensitivity against X-ray irradiation due to the lack of DBS repair in a cell culture study [25]. Therefore, we hypothesize that defects in Ku70 nuclear import accelerate DSB formation.

As DSB occur in other disease conditions without nuclear breakdown, additional factors may be involved in the nuclear changes seen in s-IBM. There is evidence that nuclear envelope dysfunction can cause both mechanical fragility of the nucleus and DNA damage. Lamins are proteins of nuclear intermediate filaments that comprise the lamina, the meshwork supporting inner nuclear membranes. Mutations in the genes that encode lamins and emerin (a lamin-associated protein) cause Emery–Dreifuss muscular dystrophy and a number of different diseases collectively called laminopathies [26]. In several laminopathies, blebbing of the nuclei in cultured fibroblasts can be seen, and it is hypothesized that such mutations result in fragile and mechanically unstable nuclei [27]. Emerin mutations can cause myopathy with rimmed vacuoles [28,29]. Besides structural integrity, the lamina is also involved in

various other processes, such as replication and gene transcription, which are intimately associated with DNA damage repair. Accordingly, impaired DNA repair has been found in several laminopathies. Fibroblasts possessing a laminopathy mutation show an excessive amount of unrepaired DNA damage, as evidenced by γ-H2AX immunohistochemistry [30]. Furthermore, lamins are important in the spatial rearrangement of nuclear pore complexes and therefore nuclear protein transport. Nuclear protein import is reduced in cells expressing lamin A mutants [31]. In the current study, we detected figures suggestive of impaired nuclear import of Ku70. Defects of nuclear import have been suggested for the mechanism of cytoplasmic accumulation of enzymes (e.g., ERK [32] and MKP-1 [33]) and nuclear molecules (e.g., pElk-1 [5,32] and TDP-43 [34]) in s-IBM. In summary, dysfunctional lamins can explain the nuclear breakdown, accumulation of DSB, and impaired nuclear transport observed in s-IBM. A specific stressor predicted in this disease [35] may affect lamins or other nuclear envelope components. Alternatively, the nuclear envelope might become fragile by aging. Cell nuclei from old individuals exhibit defects similar to those of cells from Hutchinson–Gilford progeria syndrome, which is caused by mutations of lamin A [36]. Likewise, nuclear pore complexes are not turned over in differentiated cells, and age-related alterations in nuclear pore complexes affect nuclear integrity [37]. Moreover, several studies have indicated an age-dependent decline in DNA repair capacity [38]. We suspect that these age-associated changes in nuclear envelope integrity and DNA repair mechanisms may predispose the muscles of the elderly to s-IBM pathology. In this context, the initial inducer of DSB in s-IBM muscle may be the same as that in polymyositis.

We found products that were positive for the lysosome marker LAMP-2 in rimmed vacuoles, indicating that they also originate from lysosomes. Moreover, we found that the LAMP-2-positive products were frequently associated with emerin. These findings suggest the induction of autophagy to process broken-down nuclei. In the muscle of laminopathy patients and emerin-null mice, it has been shown that autophagosomes/autolysosomes are involved in the degradation of damaged nuclear components [39].

4.2. Findings in other diseases

Recent studies indicate an up-regulation of type 1 interferon inducible proteins in dermatomyositis muscle with perifascicular atrophy [40]. A prolonged stimulation of type 1 interferon (β-interferon) induces ROS and DNA damage response in culture study [41]. Therefore, the strong myonuclear γ-H2AX staining and excessive levels of ROS found in perifascicular atrophy might correspond with this hypothesis. The strong myonuclear γ-H2AX staining found in ragged red fibers may have been induced by increased ROS caused by mitochondrial dysfunction. Although the small angulated fibers show positive γ-H2AX reactivity, the majority of these fibers were negative for HNE and iNOS. Our results suggest that γ-H2AX histochemistry is more sensitive to detect ROS injury than the two markers or that other genotoxic stresses attack on muscle cells during degeneration. Contrary to the case in s-IBM, vacuoles in OPMD, which may originate from the nucleus and show some histone H1-positivity [5], were negative for γ-H2AX. This result suggests that simple nuclear breakdown does not induce DSB. We found a strong γ-H2AX reaction in a proportion of cells in inflammatory exudates. As DSB occurs during V(D)J recombination in lymphocyte development [11], γ-H2AX-positive cells may be active in gene recombination.

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References

[1] Dalakas MC. Sporadic inclusion body myositis–diagnosis, pathogenesis and therapeutic strategies. Nat Clin Pract Neurol 2006;2:437–47.
[2] Needham M, Mastaglia FL. Sporadic inclusion body myositis: a continuing puzzle. Neuromuscul Disord 2008;18:6–16.
[3] Nalbantoglu J, Karpati G, Carpenter S. Conspicuous accumulation of a single-stranded DNA binding protein in skeletal muscle fibers in inclusion body myositis. Am J Pathol 1994;144:874–82.
[4] Greenberg SA, Pinkus JL, Amato AA. Nuclear membrane proteins are present within rimmed vacuoles in inclusion-body myositis. Muscle Nerve 2006;34:406–16.
[5] Nakano S, Shinde A, Fujita K, Ito H, Kusaka H. Histone H1 is released from myonuclei and present in rimmed vacuoles with DNA in inclusion body myositis. Neuromuscul Disord 2008;18:27–33.
[6] Lee Y, McKinnon PJ. Responding to DNA double strand breaks in the nervous system. Neuroscience 2007;145:1365–74.
[7] Narciso L, Fortini P, Pajalunga D, et al. Terminally differentiated muscle cells are defective in base excision DNA repair and hypersensitive to oxygen injury. Proc Natl Acad Sci U S A 2007;104:17010–5.
[8] Nakamura A, Sedelnikova OA, Redon C, et al. Techniques for gamma-H2AX detection. Methods Enzymol 2006;409:236–50.
[9] Kinner A, Wu W, Staudt C, Iliakis G. Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. Nucleic Acids Res 2008;36:5678–94.
[10] Dickey JS, Redon CE, Nakamura AJ, Baird BJ, Sedelnikova OA, Bonner WM. H2AX: functional roles and potential applications. Chromosoma 2009;118:683–92.
[11] O'Driscoll M, Jeggo PA. The role of double-strand break repair–insights from human genetics. Nat Rev Genet 2006;7:45–54.
[12] Mahaney BL, Meek K, Lees-Miller SP. Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. Biochem J 2009;417:639–50.
[13] Mari PO, Florea BI, Persengiev SP, et al. Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. Proc Natl Acad Sci U S A 2006;103:18597–602.
[14] Weterings E, Chen DJ. DNA-dependent protein kinase in nonhomologous end joining: a lock with multiple keys? J Cell Biol 2007;179:183–6.
[15] Hutchinson DO. Inclusion body myositis: abnormal protein accumulation does not trigger apoptosis. Neurology 1998;51:1742–5.
[16] Griggs RC, Askanas V, DiMauro S, et al. Inclusion body myositis and myopathies. Ann Neurol 1995;38:705–13.

[17] Dalakas MC, Hohlfeld R. Polymyositis and dermatomyositis. Lancet 2003;362:971–82.
[18] Nakano S, Shinde A, Ito H, Ito H, Kusaka H. Messenger RNA degradation may be inhibited in sporadic inclusion body myositis. Neurology 2005;65:420–5.
[19] Mizoguchi A, Yano Y, Hamaguchi H, et al. Localization of Rabphilin-3A on the synaptic vesicle. Biochem Biophys Res Commun 1994;202:1235–43.
[20] Pinato S, Scandiuzzi C, Arnaudo N, Citterio E, Gaudino G, Penengo L. RNF168, a new RING finger, MIU-containing protein that modifies chromatin by ubiquitination of histones H2A and H2AX. BMC Mol Biol 2009;10:55.
[21] Yang CC, Alvarez RB, Engel WK, Askanas V. Increase of nitric oxide synthases and nitrotyrosine in inclusion-body myositis. Neuroreport 1996;8:153–8.
[22] Uchida K. A lipid-derived endogenous inducer of COX-2: a bridge between inflammation and oxidative stress. Mol Cells 2008;25:347–51.
[23] Tsuruta Y, Furuta A, Furuta K, Yamada T, Kira J, Iwaki T. Expression of the lysosome-associated membrane proteins in myopathies with rimmed vacuoles. Acta Neuropathol 2001;101:579–84.
[24] Kumamoto T, Ueyama H, Tsumura H, Toyoshima I, Tsuda T. Expression of lysosome-related proteins and genes in the skeletal muscles of inclusion body myositis. Acta Neuropathol 2004;107:59–65.
[25] Okui T, Endoh D, Kon Y, Hayashi M. Deficiency in nuclear accumulation of G22p1 and Xrcc5 proteins in hyper-radiosensitive Long–Evans Cinnamon (LEC) rat cells after X irradiation. Radiat Res 2002;157:553–61.
[26] Capell BC, Collins FS. Human laminopathies: nuclei gone genetically awry. Nat Rev Genet 2006;7:940–52.
[27] Goldman RD, Shumaker DK, Erdos MR, et al. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson–Gilford progeria syndrome. Proc Natl Acad Sci U S A 2004;101:8963–8.
[28] Paradas C, Márquez C, Gallardo E, et al. X-linked Emery–Dreifuss muscular dystrophy and vacuoles: an immunohistochemical characterization. Muscle Nerve 2005;32:61–5.
[29] Fidziańska A, Rowińska-Marcińska K, Hausmanowa-Petrusewicz I. Coexistence of X-linked recessive Emery–Dreifuss muscular dystrophy with inclusion body myositis-like morphology. Acta Neuropathol 2004;107:197–203.
[30] Liu B, Wang J, Chan KM, et al. Genomic instability in laminopathy-based premature aging. Nat Med 2005;11:780–5.
[31] Busch A, Kiel T, Heupel WM, Wehnert M, Hübner S. Nuclear protein import is reduced in cells expressing nuclear envelopathy-causing lamin A mutants. Exp Cell Res 2009;315:2373–85.
[32] Nakano S, Shinde A, Kawashima S, Nakamura S, Akiguchi I, Kimura J. Inclusion body myositis: expression of extracellular signal-regulated kinase and its substrate. Neurology 2001;56:87–93.
[33] Nakano S, Shinde A, Ito H, Ito H, Kusaka H. MAP kinase phosphatase-1 is induced in abnormal fibers in inclusion body myositis. Neurology 2003;61:322–6.
[34] Salajegheh M, Pinkus JL, Taylor JP, et al. Sarcoplasmic redistribution of nuclear TDP-43 in inclusion body myositis. Muscle Nerve 2009;40:19–31.
[35] Banwell BL, Engel AG. AlphaB-crystallin immunolocalization yields new insights into inclusion body myositis. Neurology 2000;54:1033–41.
[36] Scaffidi P, Misteli T. Lamin A-dependent nuclear defects in human aging. Science 2006;312:1059–63.
[37] D'Angelo MA, Raices M, Panowski SH, Hetzer MW. Age-dependent deterioration of nuclear pore complexes causes a loss of nuclear integrity in postmitotic cells. Cell 2009;136:284–95.
[38] Gorbunova V, Seluanov A, Mao Z, Hine C. Changes in DNA repair during aging. Nucleic Acids Res 2007;35:7466–74.
[39] Park YE, Hayashi YK, Bonne G, et al. Autophagic degradation of nuclear components in mammalian cells. Autophagy 2009;5:795–804.
[40] Salajegheh M, Kong SW, Pinkus JL, et al. Interferon-stimulated gene 15 (ISG15) conjugates proteins in dermatomyositis muscle with perifascicular atrophy. Ann Neurol 2010;67:53–63.
[41] Moiseeva O, Mallette FA, Mukhopadhyay UK, Moores A, Ferbeyre G. DNA damage signaling and p53-dependent senescence after prolonged beta-interferon stimulation. Mol Biol Cell 2006;17:1583–92.

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CORRESPONDENCE

Myotonic dystrophy type 2 is rare in the Japanese population

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Myotonic dystrophy (DM) is the most common form of adult-onset muscular dystrophy and is characterized by autosomal dominant progressive myopathy, myotonia and multiorgan involvement. There are two distinct entities currently known: DM type 1 (DM1) and type 2 (DM2). DM2 is caused by the expansion of a tetranucleotide CCTG repeat in the first intron of the zinc finger protein 9 (*ZNF9*) gene on chromosome 3q21,[1] whereas DM1 is caused by a CTG repeat expansion in the 3′-untranslated region of the dystrophia myotonica-protein kinase gene (*DMPK*).[2] In the normal allele for *ZNF9*, the repeat sequence is a complex motif with an overall configuration of $(TG)_n(TCTG)_n(CCTG)_n$. The number of CCTG repeats is <30 in the normal allele, with interruptions by GCTG and/or TCTG motifs, and this allele is stably transmitted from one generation to the next.[1,3] However, in the expanded allele only the CCTG tract elongates and no GCTG and TCTG interruptions occur. The expanded *ZNF9* allele is extremely unstable and the size is highly variable, ranging from 75 to 11 000 repeats, with a mean of 5000 CCTG repeats. This unprecedented repeat size and somatic heterogeneity make the molecular diagnosis of DM2 difficult, and explain why the expansion yields variable clinical phenotypes.[4]

To date, DM2 mutations have been identified predominantly in European Caucasians.[3,5] Although a small number of DM2 mutations have been reported in non-European populations, including families in Morocco, Algeria, Lebanon, Afghanistan and Sri Lanka,[6,7] all reported that DM2 patients had been considered to originate from a single common founder because they shared an identical haplotype.[3,5,7] However, in 2008 we identified the first case of DM2 in an East-Asian population, in a Japanese patient with a disease haplotype distinct from that shared among Caucasians, indicating that DM2 exists in non-Caucasian populations and that there may have been separate founders.[8]

Thus, it was of interest to determine the frequency of DM2 in non-Caucasian populations. We studied a Japanese population for the presence of the DM2 mutation. We included both patients with clinically and/or electrically confirmed myotonia in which the DM1 mutation had been excluded and patients with the limb-girdle muscular dystrophy (LGMD) phenotype, because DM2 is generally proximal dominant[4] and the phenotype often lacks myotonia,[9] similar to LGMD, a heterogenous group of muscle disorders for which >60% of the genetic causes have remained undisclosed in Japan (Y.K. Hayashi *et al.*, unpublished data). It has been currently reported that the frequency of the DM2 mutation is more than DM1 in the European population:[10] 1 in 1830 in the general Finnish population, 1 in 988 Finnish patients with non-myotonic neuromuscular diseases and 1 in 93 Italian patients with undetermined non-myotonic proximal myopathy or asymptomatic hyperCKemia. Both the Finnish and Italian population are expected to be a relatively representative European population with regard to the DM2 mutation, because of a single European founder haplotype.[3,5,7]

Genomic DNA was extracted from blood leukocytes or muscle biopsy samples according to the standard protocols. The CCTG repeat size was determined by PCR, using primers flanking the repeat. When a single allele was amplified, Southern blot analysis using *Eco*RI or repeat-primed PCR specific for the DM2 expansion[1,4] was performed to distinguish homozygosity from heterozygosity involving a large CCTG expansion. All subjects included in this study gave informed consent and the protocol was approved by the Ethical Committee of Okayama University, Nagoya University and the National Center of Neurology and Psychiatry. In total, we studied 153 unrelated patients. In all, 34 were myotonic patients without the DM1 mutation and 119 showed a LGMD phenotype without identified LGMD mutations. Clinical

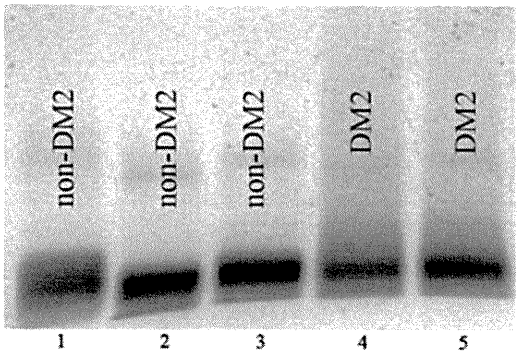

Figure 1 Repeat-primed PCR analysis. Expanded CCTG repeats in the two DM2 patients (Caucasian and Japanese DM2[9] in lanes 4 and 5, respectively) are detected as a continuous characteristic smear of products at higher molecular weight than those in non-DM2 patients (3 different individuals from the 11 patients showing a single allele by PCR amplification of the DM2 repeat in lanes 1–3).

information was assessed based on records provided by the physicians.

We identified 295 alleles ranging in length from 180 to 258 bp by PCR amplification of the DM2 repeat. Heterozygosity was identified in 142 individuals (0.93). In the remaining 11 samples showing a single allele, Southern blot or repeat-primed PCR analysis showed no expanded CCTG repeats (Figure 1), indicating that all of them are homozygous for a single allele. Thus, in our extensive survey, no DM2-related CCTG expansion was detected.

Most DM patients in Japan have been considered to have DM1 (NIH Genetics Home Reference, http://ghr.nlm.nih.gov/condition/myotonic-dystrophy). Our study confirms that DM2 is an extremely rare cause of myotonic and/or LGMD patients in Japan. Although the spectrum of clinical presentation of DM2 is variable and only one Japanese DM2 patient has been reported to date, our data have important implications concerning the indications for genetic testing and counseling for DM2 in East-Asian populations. The origin of most DM2 mutations is estimated to be 200–540 generations ago in Europe, and DM2 has since spread into several European populations.[3] The rarity of DM2 in East-Asian populations may be because of a lack of founder effects or extinction of DM2 by genetic drift or selective causes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Liquori, C. L., Ricker, K., Moseley, M. L., Jacobsen, J. F., Kress, W ., Naylor, S. L. *et al.* Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science* **293,** 864–867 (2001).
2 Harper, P. S. in *Myotonic Dystrophy* (W.B. Saunders, London, 2001).
3 Liquori, C. L., Ikeda, Y ., Weatherspoon, M., Ricker, K., Schoser, B. G., Dalton, J. C. *et al.* Myotonic dystrophy type 2: human founder haplotype and evolutionary conservation of the repeat tract. *Am. J. Hum. Genet.* **73,** 849–862 (2003).
4 Day, J. W., Ricker, K., Jacobsen, J. F., Rasmussen, L. J., Dick, K. A., Kress, W. *et al.* Myotonic dystrophy type 2: molecular, diagnostic and clinical spectrum. *Neurology* **60,** 657–664 (2003).
5 Bachinski, L. L., Udd, B., Meola, G., Sansone, V., Bassez, G., Eymard, B. *et al.* Confirmation of the type 2 myotonic dystrophy $(CCTG)_n$ expansion mutation in patients with proximal myotonic myopathy/proximal myotonic dystrophy of different European origins: a single shared haplotype indicates an ancestral founder effect. *Am. J. Hum. Genet.* **73,** 835–848 (2003).
6 Udd, B., Meola, G., Krahe, R., Thornton, C., Ranum, L., Day, J. *et al.* Report of the 115th ENMC workshop: DM2/PROMM and other myotonic dystrophies: 3rd Workshop. *Neuromuscul. Disord.* **13,** 589–596 (2003).
7 Coenen, M. J., Tieleman, A. A., Schijvenaars, M. M., Leferink, M., Ranum, L. P., Scheffer, H. *et al.* Dutch myotonic dystrophy type 2 patients and a North-African DM2 family carry the common European founder haplotype. *Eur. J. Hum. Genet.* **19,** 567–570 (2011).
8 Saito, T., Amakusa, Y., Kimura, T., Yahara, O., Aizawa, H., Ikeda, Y. *et al.* Myotonic dystrophy type 2 in Japan: ancestral origin distinct from Caucasian families. *Neurogenetics* **9,** 61–63 (2008).
9 Young, N. P., Daube, J. R., Sorenson, E. J. & Milone, M. Absent, unrecognized, and minimal myotonic discharges in myotonic dystrophy type 2. *Muscle Nerve* **41,** 758–762 (2010).
10 Suominen, T., Bachinski, L. L., Auvinen, S., Hackman, P., Baggerly, K. A., Angelini, C. *et al.* Population frequency of myotonic dystrophy: higher than expected frequency of myotonic dystrophy type 2 (DM2) mutation in Finland. *Eur. J. Hum. Genet.* **19,** 776–782 (2011).

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ORIGINAL ARTICLE

A dysphagia study in patients with sporadic inclusion body myositis (s-IBM)

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Abstract The nature of the swallowing impairment in patients with sporadic inclusion body myositis (s-IBM) has not been well characterized. In this study, we examined ten consecutive s-IBM patients using videofluoroscopy (VF) and computed pharyngoesophageal manometry (CPM). The patients were divided into two groups: patients with complaint and without complaint of dysphagia. VF results indicated pharyngeal muscle propulsion (PP) at the hypopharyngeal and upper esophagus sphincter (UES) in all s-IBM patients. Patients without complaint of dysphagia showed a mild degree of PP, whereas a severe form of PP was observed in patients with complaint of dysphagia. CPM revealed that negative pressure during UES opening was not observed in the s-IBM patients with complaint of dysphagia. Incomplete opening and PP at the UES were observed in all s-IBM patients. These results indicate that the dysphagic processes occur subclinically in s-IBM patients who may not report swallowing impairments.

Keywords Inclusion body myositis · Videofluoroscopy · Pharyngoesophageal manometry · Pharyngeal muscle propulsion · Upper esophagus sphincter

Introduction

Sporadic inclusion body myositis (s-IBM) is an inflammatory myopathy characterized by selectivity of muscle involvement, finger flexor and/or quadriceps femoris involvement, moderate elevation of muscle enzyme concentrations, and a progressive corticosteroid-resistant course. Muscle histopathology shows rimmed-vacuoles, groups of atrophic angular fibers, and endomysial mononuclear cell infiltrations.

Dysphagia has been reported in s-IBM patients. As described by Lotz et al. [1], 10% of the s-IBM patients complained of dysphagia at onset, and 40% of the patients suffered from dysphagia at the time of diagnosis. Patients with progressive dysphagia have a significantly worse functional class rating and poorer quality of life than patients with non-progressive dysphagia [2]. However, the nature of the swallowing impairment in s-IBM and other inflammatory myopathies has not been well characterized. Previous studies suggest that improper contraction of the pharyngeal muscles or cricopharyngeal muscle dysfunction may result in functional obstruction due to dysphagia [3–6].

The purpose of this study was to assess the frequency and nature of dysphagia in s-IBM patients and to identify a possible therapy for dysphagia associated with s-IBM.

Methods

Study subjects were ten consecutive patients (mean age 70.5 ± 7.1 years; 5 males and 5 females) who fulfilled the proposed diagnostic criteria for s-IBM [7] at the Department of Neurology in Wakayama Medical University between January 2000 and July 2011. Muscle biopsy studies were performed on the quadriceps femoris or biceps

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brachii in all patients. All specimens were frozen rapidly in isopentane that was chilled in dry ice, and the specimens were stored at −80°C before examination. 5-micron serial sections of each specimen were stained with hematoxylin and eosin (H&E) and modified Gomori Trichrome stain. Muscle biopsy results showed mononuclear cell infiltration around non-necrotic fibers and rimmed-vacuoles in all patients. Swallowing problems were assessed by a personal structured interview, videofluoroscopy (VF) and computed pharyngoesophageal manometry (CPM). All subjects provided written informed consent to the procedures in this study and the ethics committee at the Wakayama Medical University approved all methods used in the study.

Videofluoroscopy (VF)

All ten patients underwent oropharyngeal videofluoroscopic swallowing examination. Patients were placed upright, and the oropharynx was viewed in lateral and anterior-posterior projections. 3 ml of liquid barium and paste barium were administered by teaspoon. Swallowing examinations were repeated in different upright positions. Dysphagia severity was scored using the 8-point Penetration Aspiration Scale (PAS) [8].

Computed pharyngoesophageal manometry (CPM)

CPM was performed in all ten patients. For CPM, a sequential computer manometry system (PC polygraph) (Medtronic, Medtronic Parkway, Minneapolis) with a 4-intraluminal pressure transducer assembly (Mui Scientific, Mississauga, Ontario) was used with the recording sites set at 5 cm apart. The assembly was placed transnasally, and recording sites were chosen at the following four points: oropharynx, hypopharynx, upper esophageal sphincter (UES), and proximal esophagus (Fig. 3a). We evaluated UES pressure and pharyngeal and esophageal peristalsis during barium swallowing.

Results

Clinical findings

Ten patients were examined in this study. The subjects were divided into two groups: patients with complaint of dysphagia (Group A) and patients without complaint of dysphagia (Group B) (Table 1). Group A consisted of three men and two women who complained of dysphagia in the form of regurgitation of liquids and problems with solids. Group B consisted of two men and three women who did not complain of dysphagia. Mean age at examination was 73.8 ± 6.8 years in Group A and 67.2 ± 5.7 years in Group B. The average duration of the disease was 11.0 ± 5.4 years in Group A and 11.0 ± 1.3 years in Group B. There were no statistical differences in mean age, duration of disease and creatine kinase levels between the two groups. Other than one woman (Patient 3) in Group A, all of the patients used a cane or caster walker when walking.

Videofluoroscopy (VF)

Videofluoroscopy results indicated that all patients had a normal oral phase of swallowing and abnormalities in the pharyngeal phase. While barium material did not enter the airway in all patients in Group B, the barium material entered the airway, remained above the vocal folds, and

Table 1 s-IBM patient profiles

Patient	Sex	Complaint of dysphagia	PAS	Age	Duration of disease (years)	Aid for walking	CK (IU/L)	Complications
Group A				73.8 ± 6.8	11.0 ± 5.4		432	
1	M	(+)	2	74	14	Cane	691	HT
2	F	(+)	2	79	12	Walker	482	HT, LS
3	F	(+)	2	64	6	None	353	Sjogren Synd
4	M	(+)	2	83	4	Walker	186	Hepatitis C
5	M	(+)	2	69	19	Cane	447	HT
Group B				67.2 ± 5.7	11.0 ± 1.3		492	
6	M	(−)	1	77	12	Cane	482	HT, DM
7	F	(−)	1	62	9	Cane	503	HT
8	M	(−)	1	62	12	Cane	482	None
9	F	(−)	1	70	12	Cane	643	None
10	F	(−)	1	65	10	Cane	350	None

HT Hypertension, *LS* Lumbar spondylosis, *DM* Diabetes mellitus

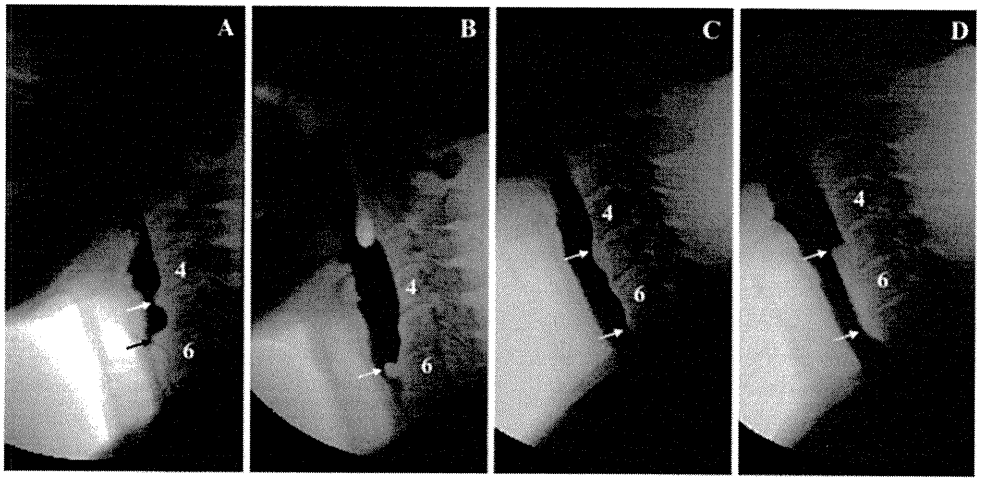

Fig. 1 Videofluoroscopic study in s-IBM patients using paste barium (**a**, **c**) and liquid barium (**b**, **d**). Pharyngeal muscle propulsions (PP) (*arrows*) were observed in Patient 2 (**a**, **b**) and Patient 4 (**c**, **d**). The degree of PP and the narrowing at the upper esophageal sphincter region in Patient 2 is more severe than that in Patient 4. The sites and shapes varied between using paste or liquid barium (**a**, **b** and **c**, **d**)

was ejected from the airway in all patients in Group A. The 8-point PAS showed a score of 2 in all patients in Group A and 1 in all patients in Group B (Table 1). Pharyngeal phase abnormalities included decreased epiglottic deflection and residue in the epiglottic vallecula and piriform recesses. Pharyngeal muscle propulsion (PP) was indicated at the UES in all ten patients without reference to dysphagia (Figs. 1, 2; Table 2). Although, the PP sites in VF ranged from C3 to C7 vertebral levels, PP shapes and sites varied between using liquid and paste barium (Figs. 1a–d, 2a, b). Patients in Group B showed a mild degree of PP, whereas a severe form of PP was observed in all five patients in Group A. An insufficiency of the UES opening was also observed in Group A patients. In addition, prominence of a segment of the hypopharyngeal sphincter muscles was observed in Patient 1 in Group A (Fig. 2a; Table 2).

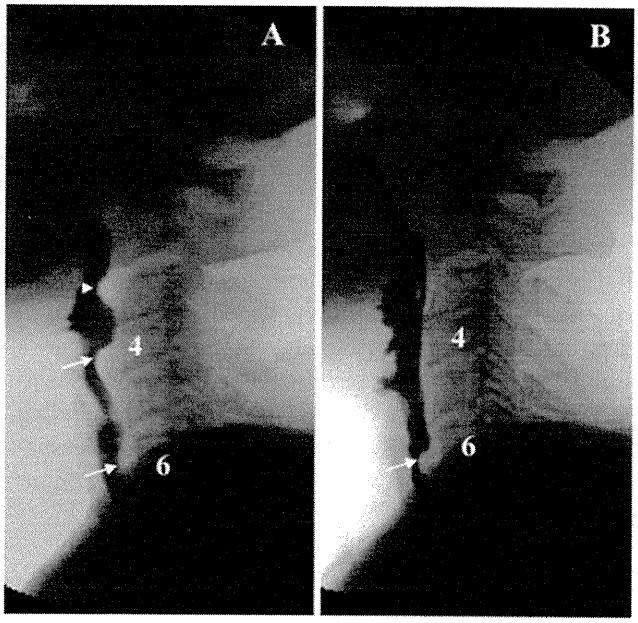

Fig. 2 Videofluoroscopic study in s-IBM patients using paste barium (**a**) and liquid barium (**b**). Pharyngeal muscle propulsions (PP) (*arrows*) and a cephalad prominence (CP) (*arrow head*) were observed in Patient 1 (**a**, **b**). The sites and shapes of PP and CP varied between using paste or liquid barium (**a**, **b**)

Computed pharyngoesophageal manometry (CPM)

In the normal control subjects, the pharyngeal peak pressure at the oropharynx and hypopharynx elevated simultaneously (Fig. 3b-1, 2). The pharyngeal pressure at the oropharynx was higher than that at the hypopharynx. Contrary to the high pharyngeal pressure in the oropharynx and hypopharynx, the pressure at the UES decreased until the UES opened (nadir deglutitive UES pressure) (Fig. 3b-3, arrow). After the barium paste passed through the entrance of the UES, pharyngeal pressure at the UES increased and pushed the paste to the upper esophagus (Fig. 3b-3).

In the s-IBM patients, the pressure at the oropharynx and hypopharynx was very low compared with that of the normal controls (Fig. 3c-1, 2). In addition, the negative pressure during UES opening (nadir deglutitive UES pressure) observed in the normal controls was not observed in the s-IBM patients with dysphagia (Fig. 3c-3). Manometric recordings in all s-IBM patients revealed a lack of oropharyngeal peristaltic activity, a decreased hypopharyngeal peristalsis, and a reduced peak of post-deglutitive UES pressure, while the esophageal resting pressure was normal. In addition, all s-IBM patients in Group A demonstrated no deglutitive UES relaxation in the CPM study.

Table 2 Videofluoroscopic and manometric findings in ten patients with s-IBM

Pt	Group	Videofluoroscopy			
		PP site	Insufficiency of UES opening	Pooling site of barrium	Manometry
1	A	C3, C4-5, C6-7	(+)	EV, piriform	No UES relaxation
2		C4-5, C5-6, C6	(+)	EV, piriform	Decreased oro-hypopharyngeal pressure
3		C5-6, C6-7	(+)	EV	Decreased deglutitive UES pressure
4		C5-6	(+)	EV, piriform	
5		C6-7	(++)	EV, piriform	
6	B	C3-4	(−)	EV	Incomplete UES relaxation
7		C5-7	(−)	None	Decreased oro-hypopharyngeal pressure
8		C5-6	(−)	EV, piriform	Decreased deglutitive UES pressure
9		C4-5, C6-7	(−)	None	Incomplete UES relaxation
10		C5-6	(−)	EV	Incomplete UES relaxation

PP pharyngeal muscle propulsion, *UES* upper esophageal sphincter, E*V* epiglottic vallecula, *piriform* piriform recess

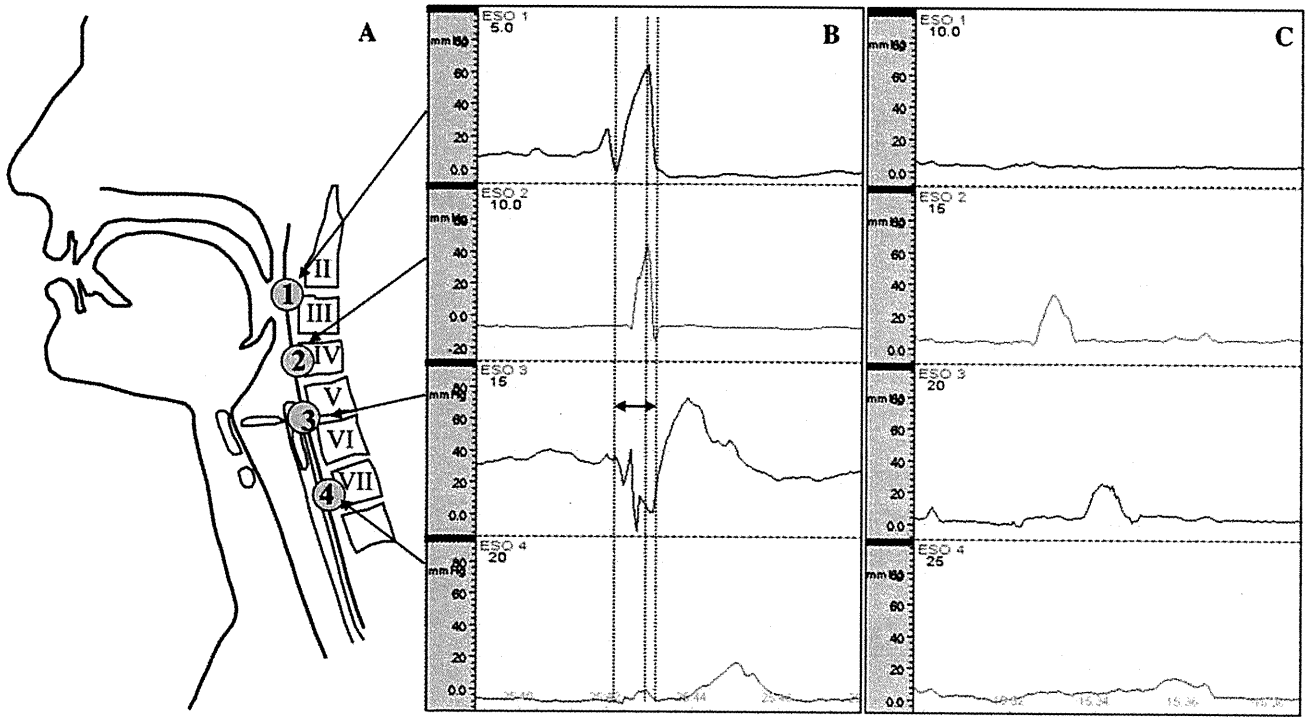

Fig. 3 Manometry **a** Manometry study using simultaneous 4-channel pressure recording during 3 ml barium swallowing. **b** Manometric findings in a healthy control subject: *channel 1* oropharynx, *channel 2* hypopharynx, *channel 3* UES, *channel 4* proximal esophagus. **c** Patient 3: Pharyngoesophageal manometry during swallowing solid barium paste. The pressure at the oropharyngx, hypopharyngx and UES is none or very low compared with that of normal controls. UES negative pressure during UES opening (nadir deglutitive UES pressure), which was observed in normal controls (**b**, *arrow*), was not observed

In contrast, incomplete UES relaxation was observed in Group B patients (Table 2).

Discussion

The consequences of dysphagia include weight loss, the need for modified food consistency and non-oral feeding. Pulmonary infections occur in patients with dysphagia, and aspiration pneumonia is considered a main cause of death in s-IBM patients. We observed PP at the UES and/or hypopharyngeal muscle in all ten s-IBM patients. PP was first reported as a prominent cricopharyngeal impression at the cricopharyngeal muscles [9], and these findings were observed not only in s-IBM patients, but also in mitochondria myopathy patients [10]. PP is also defined as

cricopharyngeal achalasia. We observed PP at the hypopharyngeal muscles in Patient 1, and this PP has been named cephalad prominence [11]. Because PP in VF is observed in front of the vertebral discs, it may be considered the result of disc prolapse. In this study, the PP shapes and sites varied between using liquid and paste barium. Therefore, we conclude that the PP observed here did not represent the result of disc prolapse.

PP was observed at the UES in all ten s-IBM patients, while only five patients (Group A) complained of dysphagia. The local esophageal diameter reduction by PP in Group A was >50% at the UES during swallowing. The degree of PP in Group A was more severe than that of Group B (Fig. 1a–d). In addition, the five patients in Group A showed insufficiency of the UES opening in VF. PP is revealed as barium manages to go through the non-extended pharynx. It represents improper dilation of the pharyngeal muscles during barium passing. A dilation problem of the pharyngeal muscles can occur with the patient being unaware of swallowing difficulties. Although, there was a positive relationship between the severity of PP and the insufficiency of UES opening, PP does not induce cricopharyngeal obstruction. PP represents only the result of dilation problems of pharyngeal musculature.

The lack of negative UES pressure (nadir deglutitive UES pressure) in the s-IBM patients with dysphagia may explain swallowing difficulties. Incomplete pharyngeal musculature opening at the UES during swallowing may have a number of different causes, including impaired relaxation or spasm of the UES, hyperplasia and hypertrophy or fibrosis of the cricopharyngeal muscles, weakness of the suprahyoid muscles, and failure of neural inhibition of tonic sphincter contraction [12]. An examination of cricopharyngeal muscle biopsies of one patient was reported at the time of cricopharyngeal myotomy [4, 13]. Numerous small, round atrophic muscle fibers were observed which varied in size. Because the cricopharyngeal muscles have a sphincteric function, atrophic cricopharyngeal muscles failed to push foods toward the upper esophagus. In our manometry study, post-deglutitive UES pressure was observed, but the peak pressure was greatly reduced. Low pressure at the oropharynx and hypopharynx, and hypo-oropharyngeal peristaltic activities induced problems with the propulsion of the bolus through the sphincter muscles. In addition, there was a marked increase in endomysial connective tissue, some replacement by fat, and proliferative connective tissue. In s-IBM patients in Group A, nadir deglutitive UES pressure was not observed. This finding suggests that endomysial proliferative connective tissue prevented the extension and relaxation of the UES.

The prevalence of s-IBM in Asian populations including Japan has not been examined. Recently, a national survey study revealed that the number of s-IBM patients in Japan is estimated to be around 1,250 and that the prevalence of s-IBM is 9.83 per million [14]. We examined ten patients, representing 0.8% of all s-IBM patients in Japan. Although the number of patients was very low and results of our study are limited in significance, our study revealed a tendency in s-IBM patients with dysphagia.

Recently, cricopharyngeal myotomy was selected to reduce dysphagia in s-IBM patients [15]. The aim of the myotomy is to remove the impaired UES relaxation, which is not overcome by decreased PP. Indeed, myotomy has shown to be useful in improving dysphagia associated with UES hyperactivities in s-IBM, however, other therapies, such as intravenous immunoglobulin [16] or botulinum toxin A [17], and balloon dilation have been employed for s-IBM patients with dysphagia.

The combination test using VF and manometry is needed to assess the preservation of sphincter muscle strength and the efficacy of each therapy. Deglutitive pharyngoesophageal functions should be examined routinely in all s-IBM patients even if they do not complain of dysphagia.

References

1. Lotz BP, Engel AG, Nishino H, Stevens JC, Litchy WJ (1989) Inclusion body myositis. Observations in 40 patients. Brain 112(Pt 3):727–747
2. Houser SM, Calabrese LH, Strome M (1998) Dysphagia in patients with inclusion body myositis. Laryngoscope 108:1001–1005
3. Wintzen AR, Bots GT, de Bakker HM, Hulshof JH, Padberg GW (1988) Dysphagia in inclusion body myositis. J Neurol Neurosurg Psychiatry 51:1542–1545
4. Danon MJ, Friedman M (1989) Inclusion body myositis associated with progressive dysphagia: treatment with cricopharyngeal myotomy. Can J Neurol Sci 16:436–438
5. Dietz F, Logeman JA, Sahgal V, Schmid FR (1980) Cricopharyngeal muscle dysfunction in the differential diagnosis of dysphagia in polymyositis. Arthritis Rheum 23:491–495
6. Kagen LJ, Hochman RB, Strong EW (1985) Cricopharyngeal obstruction in inflammatory myopathy (polymyositis/dermatomyositis). Report of three cases and review of the literature. Arthritis Rheum 28:630–636
7. Griggs RC, Askanas V, DiMauro S, Engel A, Karpati G, Mendell JR, Rowland LP (1995) Inclusion body myositis and myopathies. Ann Neurol 38:705–713
8. Rosenbek JC, Robbins JA, Roecker EB, Coyle JL, Wood JL (1996) A penetration-aspiration scale. Dysphagia 11:93–98
9. Seaman WB (1966) Cineroentgenographic observations of the cricopharyngeus. Am J Roentgenol Radium Ther Nucl Med 96:922–931
10. Kornblum C, Broicher R, Walther E, Seibel P, Reichmann H, Klockgether T, Herberhold C, Schroder R (2001) Cricopharyngeal achalasia is a common cause of dysphagia in patients with mtDNA deletions. Neurology 56:1409–1412
11. Shapiro J, Martin S, DeGirolami U, Goyal R (1996) Inflammatory myopathy causing pharyngeal dysphagia: a new entity. Ann Otol Rhinol Laryngol 105:331–335

12. Cook IJ, Blumbergs P, Cash K, Jamieson GG, Shearman DJ (1992) Structural abnormalities of the cricopharyngeus muscle in patients with pharyngeal (Zenker's) diverticulum. J Gastroenterol Hepatol 7:556–562
13. Verma A, Bradley WG, Adesina AM, Sofferman R, Pendlebury WW (1991) Inclusion body myositis with cricopharyngeus muscle involvement and severe dysphagia. Muscle Nerve 14:470–473
14. Suzuki N, Aoki M, Mori-Yoshimura M, Hayashi YK, Nonaka I, Nishino I (2011) Increase in number of sporadic inclusion body myositis (sIBM) in Japan. J Neurol. doi:10.1007/s00415-011-6185-8
15. St Guily JL, Moine A, Perie S, Bokowy C, Angelard B, Chaussade S (1995) Role of pharyngeal propulsion as an indicator for upper esophageal sphincter myotomy. Laryngoscope 105:723–727
16. Cherin P, Pelletier S, Teixeira A, Laforet P, Simon A, Herson S, Eymard B (2002) Intravenous immunoglobulin for dysphagia of inclusion body myositis. Neurology 58:326
17. Liu LW, Tarnopolsky M, Armstrong D (2004) Injection of botulinum toxin A to the upper esophageal sphincter for oropharyngeal dysphagia in two patients with inclusion body myositis. Can J Gastroenterol 18:397–399

IX. 神経・筋

ミオパチー

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■診断基準

ミオパチーとは筋疾患を意味する用語であり，何らかの理由で骨格筋が直接侵される疾患を指す．個々の筋疾患についての診断基準は存在するが，ミオパチー一般の診断基準は存在しない．

本稿では暫定的にミオパチーの診断基準として

①筋力低下・筋萎縮・筋痙攣または血清クレアチンキナーゼ(CK)異常を呈する．

②神経系または骨格系の異常を除外できる．

の2項目をあげたい．すなわちこれらの項目を満たした場合に，何らかの筋疾患が存在する可能性が高い．

■病型分類

ミオパチーの原因は遺伝性・感染性・代謝性・薬剤性に加えて原因不明までさまざまである．今回われわれは，過去の国内および海外での分類をもとに便宜的にTable 1のように病型分類する．以下，各病型について概説する．

1．筋ジストロフィー

筋ジストロフィーは一般的には「骨格筋の変性・壊死を主病変とし，臨床的には進行性の筋力低下をみる遺伝性の疾患である」と定義されている[1]．Duchenne/Becker型・Emery-Dreifuss型，肢帯型(LGMD)・先天性(福山型・非福山型)，顔面肩甲上腕型(FSHD)・眼咽頭型等に分類される．

Duchenne/Becker型は筋ジストロフィー全体のうち約50%を占め，四肢筋力低下による歩行障害に加え心不全など多彩な合併症があることから広く知られている．

2．筋強直症候群

筋強直症候群のうちもっとも有病率が高いのが，筋強直性ジストロフィーであり*DMPK*(dystrophia myotonica protein kinase)遺伝子の3′-非翻訳領域のCTG反復配列の伸張により発症する．筋強直性ジストロフィー2型(proximal myotonic myopathy：PROMM)は*ZNF-9*(zinc finger protein-9)遺伝子のイントロン領域のCCTG反復配列により発症する．臨床症状はきわめて多彩で，骨格筋症状(筋強直・心筋障害)のみならず，中枢神経(認知機能障害など)・内分泌(耐糖能機能異常など)および眼症状(白内障・網膜色素変性症)を合併する．

その他，先天性筋強直性ジストロフィー・先天性筋強直症(Thomsen病)・先天性パラミオトニアも筋強直症状を呈する．

3．遠位型ミオパチー

筋疾患の多くは体幹に近い四肢筋を侵すことが多いが，例外的に遠位筋優位に侵される一連の遺伝性筋疾患を遠位型ミオパチーという．神経原性筋疾患との鑑別が重要である．本邦では三好型ミオパチー・縁取り空胞を伴う遠位型ミオパチー(DMRV)・眼咽頭遠位型ミオパチーの3疾患が広く知られているが，その他Welander型遠位型ミオパチー(40代以降に発症し，手指伸筋の筋力低下を示す)・tibial muscular dystrophy(TMD)(前脛

Table 1. ミオパチーの病型分類

1．筋ジストロフィー Duchenne/Becker 型筋ジストロフィー Emery-Dreifuss 型筋ジストロフィー 肢帯型筋ジストロフィー 先天性筋ジストロフィー 顔面肩甲上腕型筋ジストロフィーなど	MERRF(ragged-red fiber を伴うミオクローヌスてんかん) CPEO(慢性進行性外眼筋麻痺)など
2．筋強直症候群 筋強直性ジストロフィー 先天性筋強直性ジストロフィー 先天性筋強直症(Thomsen 病) 先天性パラミオトニアなど	6．炎症性筋疾患 多発筋炎 皮膚筋炎 封入体筋炎など
3．遠位型ミオパチー 三好型ミオパチー 縁取り空胞を伴う遠位型ミオパチー 眼咽頭遠位型ミオパチー Welander 型遠位型ミオパチーなど	7．代謝性疾患 糖原病 VLCAD 欠損症 原発性カルニチン欠損症 多種アシル CoA 脱水素酵素欠損症など
4．先天性ミオパチー ネマリンミオパチー セントラルコア病 ミオチュブラーミオパチー 先天性筋線維タイプ不均等症など	8．内分泌性疾患 甲状腺ホルモン異常 副甲状腺ホルモン異常 副腎皮質ホルモン異常など
5．ミトコンドリア病 MELAS(高乳酸血症・卒中様症状を伴うミトコンドリア病)	9．中毒・感染性筋疾患 アルコール性ミオパチー ステロイドミオパチー スタチンミオパチー ウイルス・寄生虫感染症など
	10．周期性四肢麻痺

骨筋の筋力低下を示す)・distal *VCP*(valosin containing protein)-mutated myopathy(高齢発症で Paget 病と前頭側頭型認知症を伴う)・Miyoshi-like myopathy(三好型ミオパチーに臨床的に類似するが, dysferlin は正常)・筋原線維性ミオパチー(myofibrillar myopathy)などがある．筋原線維性ミオパチーは，病理学的な分類であり症候は多岐にわたっている．

4．先天性ミオパチー

臨床的には，①フロッピーインファント(floppy infant：生下時または乳児期早期より筋緊張低下を示す)，②頸部屈筋群の筋力低下，③顔面筋罹患(高口蓋)，④呼吸筋麻痺を呈することが多い．これらの症状に加え特徴的な筋病理所見を呈する疾患群を先天性ミオパチーと分類する．臨床経過からは生下時より呼吸障害や嚥下障害が強い重症型(severe infantile form)，発達遅延があるものの非進行性または緩徐進行性の良性先天型(benign congenital form)および成人発症型(adult onset form)の3型に分類される．診断は筋病理所見に基づいてなされる．ネマリンミオパチー・セントラルコア病・ミオチュブラーミオパチー・先天性筋線維タイプ不均等症(congenital fiber type disproportion：CFTD)などがある．

5．ミトコンドリア病

ミトコンドリア病はミトコンドリアの機能障害により何らかの症状を呈する病態であると定義できるが，通常は呼吸鎖酵素におけるエネルギー産生障害による疾患を指す．呼吸鎖は5種類の酵素複合体から構成される．酵素複合体に含まれる蛋白質はミトコンドリア DNA によりコードされるものと核 DNA によりコードされるものがある．いずれの異常によってもミトコンドリア病をきたしうる．ミトコンドリア DNA 変異は母系遺伝を

示すことがしばしばあるのに対し，核 DNA 異常は通常常染色体性遺伝を示す．

ミトコンドリア病は Table 1 に示した 3 大病型のように臨床症状により分類される場合と，生化学的な機能異常（ピルビン酸脱水素酵素複合体：PDHC 欠損症など）により分類される場合とがある．

6．炎症性筋疾患

多発筋炎・皮膚筋炎および封入体筋炎などが含まれる．

炎症性筋疾患の診断には臨床症状に加え，筋病理組織による診断が重要である．多発筋炎では，筋線維周囲および筋線維内へのリンパ球の浸潤がみられる．しばしば筋周鞘がアルカリホスファターゼ（alkaline phosphatase）染色で陽性を示す．皮膚筋炎では筋束周辺の筋線維萎縮（perifascicular atrophy）が特徴的変化として知られている．

成人の皮膚筋炎では，腫瘍性病変の合併頻度が高い．近年さまざまな自己抗体が同定されてきており，それぞれの抗体と臨床・病理学的変化の関連について整理されつつある．

封入体筋炎は高齢者にみられる疾患であり，大腿四頭筋と深指屈筋が高頻度に侵される．病理学的には，筋線維周囲へのリンパ球浸潤に加えて縁取り空胞を認めることが特徴である．

7．代謝性疾患

筋線維内でのエネルギー産生は，前述のミトコンドリアでの呼吸鎖のほかに細胞質内での解糖系や脂肪酸β酸化などに依存している．解糖系の異常で糖原病をきたす．とくに糖原病のうち，糖原病Ⅱ型（Pompe 病）では酸性α-グルコシダーゼ欠損によりライソゾーム内にグリコーゲンが蓄積し，肝細胞障害・肥大型心筋症・筋力低下を示す．近年，酵素補充療法が保険収載され，早期診断の重要性が増している．脂質代謝異常には VLCAD（very long chain acyl-CoA dehydrogenase）欠損症など横紋筋融解症をきたす疾患群のほか，原発性カルニチン欠損症や多種アシル CoA 脱水素酵素欠損症など脂質蓄積性ミオパチーをきたす疾患群がある．

8．内分泌性疾患

甲状腺ホルモン異常によりしばしば筋障害をきたす．甲状腺機能低下症では近位筋優位の筋力低下と易疲労性，緩徐な腱反射がしばしばみられる．またハンマーによる筋叩打により筋膨隆現象（mounding phenomenon）がみられる．血清 CK 値は無症候性でも高値を示すことがある．甲状腺中毒性ミオパチーは初期は近位筋の筋力低下を示すことが多く，ときに呼吸筋障害を呈することがある．血清 CK 値は正常または低下することがある．また甲状腺機能低下症・亢進症ともに重症筋無力症の合併頻度が高いことが知られている．

原発性アルドステロン症では低カリウム血症をきたしてミオパチーを呈する．後述の周期性四肢麻痺を参照されたい．

9．中毒・感染性疾患

中毒性疾患ではアルコール性ミオパチーやステロイドミオパチー，スタチンミオパチーなどの頻度が高い．アルコール性ミオパチーは急性壊死性ミオパチー・急性低カリウム性ミオパチー・慢性アルコール性ミオパチー・アルコール性心筋症といったさまざまな病型を含む．ステロイドミオパチーは下肢近位筋および下肢帯の筋力低下と筋萎縮を呈することが多い．血清 CK は正常または軽度の上昇にとどまる．スタチンミオパチーはスタチン（HMG-CoA 還元酵素阻害薬）の内服により誘発される横紋筋融解症を呈する．腎不全の合併が増悪因子となる．

感染性疾患は，主にウイルス性が多いがごくまれに寄生虫感染も報告される．ウイルス性筋炎の原因として，コクサッキー・エコー・インフルエンザ・HIV・パルボウイルスなどが知られている．

10．周期性四肢麻痺

発作性に四肢および体幹の弛緩性麻痺を呈する．内分泌異常を除外したものを原発性周期性四肢麻痺という．イオンチャネルをコードしている遺伝子変異により筋線維の膜電位異常が起こると

考えられる．発作中の血清カリウムイオン濃度により高・低カリウム性周期性四肢麻痺に分けられる．

低カリウム性周期性四肢麻痺の原因として*CACNA1S*(calcium channel, voltage-dependent, L type, alpha-1S subunit)遺伝子の異常が同定されている．高カリウム性周期性四肢麻痺の原因としては*SCN4A*(sodium channel, voltage-gated, type Ⅳ, alpha subunit)遺伝子の異常が同定されている．筋強直症候群の項であげた先天性パラミオトニアは同一の遺伝子異常による allelic な疾患である．

■重　症　度

筋力自体の評価に加えて，筋力低下による歩行障害などの日常生活動作(activities of daily living：ADL)制限・嚥下機能障害による摂食量の低下や誤嚥・呼吸筋力低下による換気障害・心筋機能低下等の評価が重要である．

筋力は，一般に徒手筋力検査(manual muscle testing：MMT)で評価される．ただし，検者間での差異があるため，ダイナモメーターやピンチメーターによる定量的評価を行うこともある．握力は MMT に比較して客観的であると考えられるが，器材による違いがある．また，加速度計による運動の評価も行われる．血清 CK 値は通常，筋線維の壊死を反映するが，進行期ではむしろ低下することに注意が必要である．CK 値の増加がみられない筋疾患もある．ミトコンドリア病では通常血清および髄液中の乳酸の上昇がみられる．筋量の評価には骨格筋 CT が用いられる．炎症性筋疾患では，炎症部位の同定に筋 MRI が有用である．

嚥下機能評価にはしばしば食道嚥下造影検査が用いられる．嚥下性肺炎の危険性評価に加え，経口栄養から経管栄養への切り替えを判断する際に有用である．

肺機能検査や動脈血ガス分析により呼吸筋障害の程度を評価する．

心臓超音波検査で心拍出量や心筋の運動量を調べて心筋障害の程度を評価する．血液生化学的検査では脳性ナトリウム利尿ペプチド(brain natriuretic peptide：BNP)が心不全の指標として有用である．

文　献

1) Walton JN, Nattrass FJ：On the classification, natural history and treatment of the myopathies. Brain **77**：169, 1954

6 筋疾患とオートファジー

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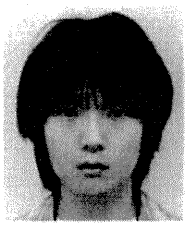

本田 真也
2008年3月東京理科大学院基礎工学研究科生物工学専攻卒業，同年4月国立精神・神経センター神経研究所疾病研究第一部流動研究員．研究テーマは，ダノン病，XMEA治療法開発に向けた分子病態の解明．趣味は，サッカー

Key words：ミオパチー，オートファジー，自己貪食空胞，遺伝性筋疾患

Abstract
筋細胞におけるオートファジーの役割，およびリソソームの機能は長らく不明であった。しかし，自己貪食空胞が蓄積する筋疾患の存在から，筋細胞におけるリソソームとオートファジーの重要性が注目されるようになっている。オートファジーの異常を示す筋疾患は，その原因遺伝子と病理観察から，①オートファジー/リソソーム系の異常によるものと，②オートファジーそのものの異常ではなく，2次的にオートファジーが惹起されるものの2つに分類することができる。

1. 骨格筋・心筋におけるオートファジー研究

正常な骨格筋・心筋においては，形態学的観察でリソソームや自己貪食空胞が認められることは少なく，骨格筋・心筋におけるオートファジーの役割，およびリソソームの機能は長らく不明のままであった。しかしながら，自己貪食空胞が蓄積する筋疾患の存在が明らかになり，筋肉におけるリソソームとオートファジーの重要性が重視されるようになってきた。我々はこれまでにオートファジーの機能異常により筋線維内に自己貪食空胞が蓄積する自己貪食空胞性ミオパチー(Autophagic vacuolar myopathy：AVM)という一群の筋疾患を提唱し，広く世界に受け入れられている。

原因遺伝子の明らかになっているAVMとして，ダノン病・X-linked myopathy with excessive autophagy (XMEA)・酸マルターゼ欠損症・縁取り空胞型遠位型ミオパチー(distal myopathy with rimmed vacuoles：DMRV)があげられる。それぞれの原因遺伝子はLAMP-2 (lysosome-associated membrane protein-2)・Vma21・酸マルターゼ・GNE（ウリジン二リン酸-N-アセチルグルコサミン2-エピメラーゼ／N-アセチルマンノサミンキナーゼ）である。LAMP-2はリソソーム膜タンパク質，Vma21はV-ATPaseの複合体形成因子，酸マルターゼはグルコース分解を行うリソソーム酵素，GNEはシアル酸合成に必須な2つの酵素をコードしている。

このようにAVMは原因遺伝子から，①オートファジー／リソソーム系そのものの機能異常により病態を示すもの，②オートファジー／リソソーム系の機能異常が本質的な疾患原因ではなく，2次的にオートファジーの働きが惹起し病態を示すものの2つに分類される。前者ではダノン病・XMEA・酸マルターゼ欠損症，後者ではDMRVが代表的な筋疾患として分類

Autophagic vacuolar myopathy: Shinya Honda and Ichizo Nishino, Department of Neuromuscular Research, National Institute of Neuroscience, NCNP

される。以下，それぞれの筋疾患に対しての病理症状およびこれまでの研究成果について記す。

2. 自己貪食空胞性ミオパチー（AVM）

1）ダノン病

ダノン病は，X連鎖優性遺伝のまれな疾患で，全世界で数十例の報告があるにすぎない。ダノン病は，X染色体上にコードされているリソソームの膜タンパク質の1つLAMP-2が欠損することにより起こる遺伝性疾患であり，精神遅滞・ミオパチー・肥大型心筋症を3徴とする。ダノン病は症状が潜行性のため早期の発見が難しいが，すべての患者で進行性の心筋症がみられる。この心筋症はしばしば不静脈を伴い突然死にいたることがあり，現在のところ心臓移植以外に効果的な治療法は報告されていない。患者の筋組織をみると，筋線維内に多数の小胞が蓄積しており，電子顕微鏡観察から，それらが自己貪食空胞であることが分かる。また，これら蓄積空胞の周囲には，それを取り囲むように筋鞘膜様の特徴をもつ膜 (autophagic vacuoles with sarcolemmal features : AVSF)が観察される（図1-A)。AVSFでは空胞膜にほぼすべての筋鞘膜タンパク質が発現しており，アセチルコリンエステラーゼ活性を有している。これら特徴からAVSF形成は，細胞が自身の内部に細胞外環境を作り出しているかのような状況であるといえる。しかしながら，現在のところ，このAVSFがどのようにして形成されるのか？病態への関与は何なのか？などといった疑問は解明されておらず，そもそもAVSF形成が，生体防御的に働いているのか，あるいは病態悪化要因であるのかさえ不明である。

LAMP-2は，その常染色体上ホモログであるLAMP-1と共に，リソソーム膜の約50%を構成するリソソーム膜主要構成タンパク質であり，ノックアウトマウスを用いた研究からリソソームの移動・オートファゴソームとの融合に関与していることが明らかになっている。LAMP-2は正常では心臓・骨格筋・脳において強く発現しており，ダノン病では，これらの領域において，恒常的におこっているオートファジーがその最終段階（オートファゴソームとリソソームの融合段階）でストップしており，症状を呈するようになると考えられる。

2） X-linked myopathy with excessive autophagy (XMEA)

XMEAはX染色体劣性遺伝形質をとる遺伝性筋疾患であり，女性保因者は発症しない。この疾患は臨床的にはダノン病よりも軽く，心筋障害もきたさないが，筋組織所見は極めて類似している。近位筋優位の進行性の筋力低下と委縮が認められるが，程度は軽く，60歳を過ぎても歩行可能な例が多い。

XMEAは長らくその原因遺伝子が不明のままであったが，2009年についにその原因遺伝子がVMA21であることが明らかになった。VMA21はV-ATPaseの複合体構成因子であり，その欠損はV-ATPaseの活性を低下させる。V-

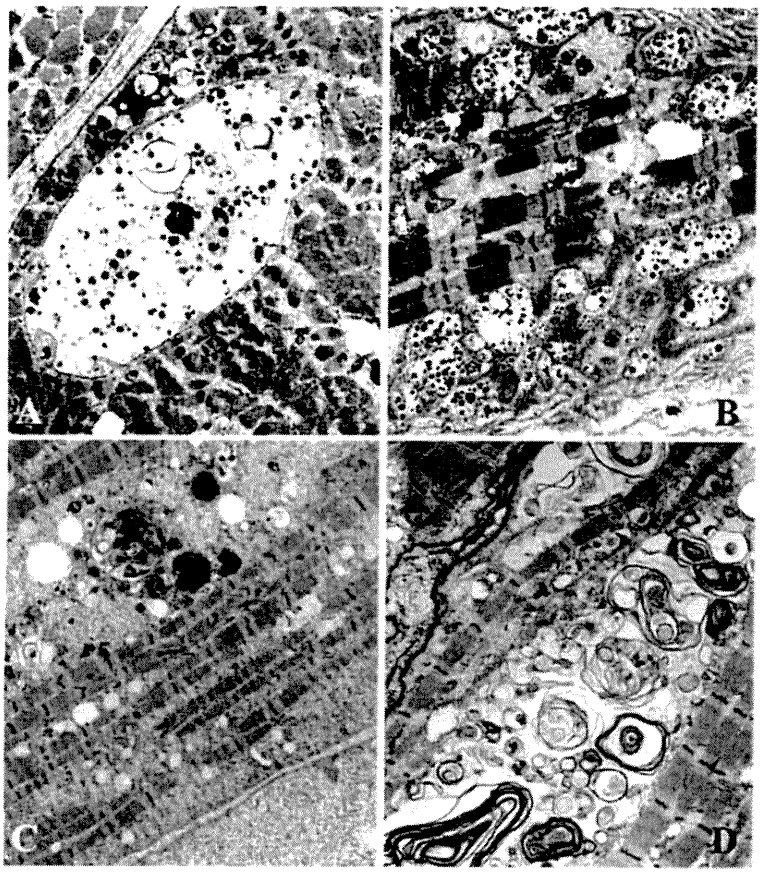

図1　AVM骨格筋の電子顕微鏡写真
A：ダノン病、B：XMEA、C：酸マルターゼ欠損症、D：DMRV

ATPaseはリソソーム内を酸性に保つためのプロトンポンプとして働いており，その機能低下によりリソソーム内のpHが上昇し，タンパク質の分解が低下する。つまり，XMEAではVMA21の変異により，リソソームの分解能が低下することで，オートファジーの機能異常がおこり病状を呈する。

XMEAの筋病理ではダノン病と同様に筋線維内にAVSFを認める。通常AVMの電子顕微鏡観察では，蓄積した自己貪食空胞内には分解前の細胞内小器官，分解された不定形の分解物のどちらかを認めるが，XMEAにおいては空胞内に電子密度の高い円い顆粒状の内容物が観察される（図1-B）。さらに，蓄積された空胞があたかもエキソサイトーシスにより細胞外に放出されているような像も観察される。また筋鞘膜部の基底膜が肥厚化し，自己貪食空胞内に見られたものと同様の細胞質分解産物の蓄積が観察される。これらの現象の意義はいまだ明らかになっていないが，この現象は，細胞が分解できずに蓄積した空胞を，エキソサイトーシスの経路に乗せかえること