Acta Neuropathol (1995) 89:29–34

REGULAR PAPER

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Muscle fiber degeneration in distal myopathy with rimmed vacuole formation

Received: 21 June 1994 / Revised: 19 August 1994 / Accepted: 29 August 1994

Abstract In 11 patients with distal myopathy with rimmed vacuole formation (DMRV), a well-known autosomal recessively inherited disorder, the rimmed vacuole formation appears to be the main pathological change accounting for the progressive muscle fiber degeneration. To gain a better understanding of the pathophysiology of the vacuole formation, we applied Congo red and immunohistochemical stains to muscle biopsies from these patients and the results were compared with those of patients with inclusion body myositis (IBM). The vacuoles in DMRV contained Congophilic amyloid material and deposits immunoreactive for β-amyloid protein, both the NH_2 and COOH termini of β-amyloid protein precursor, ubiquitin, and tau protein. These results were similar to those seen in our present cases of IBM as well as in previously reported cases. Therefore, there may be no pathogenetic differences in the formation of rimmed vacuoles in DMRV and IBM. Nevertheless, the degenerative process involved in rimmed vacuole formation in various diseases may share a common pathogenetic mechanism with that in amyloid-plaque formation in Alzheimer's disease brain as has been proposed previously.

Key words Rimmed vacuole · Distal myopathy with rimmed vacuole formation · Inclusion body myositis

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Introduction

Distal myopathy with rimmed vacuole formation (DMRV) [25, 28] is inherited through an autosomal recessive trait and is characterized clinically by preferential peroneal muscle involvement in early adulthood. Except for the rimmed vacuole formation seen predominantly in the atrophic fibers, there is no apparent muscle fiber necrosis or evidence of denervation pathologically. In addition, intranuclear and/or cytoplasmic filamentous inclusions, which are similar to those seen in inclusion body myositis (IBM) [10, 20, 30], have also been observed in patients with DMRV [22, 25]. Pathological examination of muscle itself does not always differentiate between IBM and DMRV, unless inflammatory cell infiltration is present, which is indicative of IBM. Patients with rimmed vacuoles in their muscle biopsies, even in the absence of inflammation, are occasionally given the diagnosis of IBM [10, 12]. In addition, some patients with a positive family history of muscle disease have been included among the cases of IBM [21, 24]. Askanas et al. [4, 5] coined the term hereditary inclusion body myopathy for such diseases to differentiate the disease without an inflammatory process from IBM. To determine whether DMRV undergoes a similar degenerative process in the rimmed vacuole formation as that seen in IBM and hereditary inclusion body myopathy, we examined muscle biopsies by histochemical and immunohistochemical methods.

Materials and methods

We examined muscle biopsies from 11 patients with DMRV, 4 patients with IBM and 10 normal adults. The biceps brachii muscles were biopsied from 4 patients with DMRV, all of the patients with IBM and all controls, and the rectus femoris and the gastrocnemius muscles were biopsied from 5 and 2 patients with DMRV, respectively. The diagnosis of DMRV was based on the characteristic clinical features and the absence of inflammatory cell infiltration in the muscle pathology findings. In the cases with IBM, we followed the diagnostic criteria for IBM proposed by Lotz et al. [20]: (1) no family history, (2) clinical characteristics of an inflamma-

tory myopathy, (3) a number of muscle fibers with rimmed vacuoles, (4) endomysial and autoaggressive inflammatory exudate, and (5) filamentous inclusions on electron microscopy.

Muscle biopsies were frozen in liquid nitrogen-cooled isopentane for histochemistry, and fixed with 2% glutaraldehyde for electron microscopy. Transverse serial frozen sections of 10 μm thick were stained with H&E, modified Gomori trichrome and various histochemical methods. Additional sections of these muscle biopsies were stained with Congo red and, immunohistochemically, with antibodies against β-amyloid protein (βAP), NH_2 and COOH termini of β-amyloid protein precursor (APP), ubiquitin and tau protein.

Congo red staining was performed by the method described by Mendell et al. [23]. Sections of muscle and peripheral nerve from a patient with amyloidosis served as positive controls. The sections were examined under polarized and fluorescence microscopies [6].

For immunohistochemical study of βAP, NH_2 and COOH termini of APP and tau protein, transverse sections of 5–8 μm thick were fixed in periodate-lysine-paraformaldehyde at 4°C. The sections were incubated in 1:300 diluted rabbit polyclonal antibodies against βAP, NH_2 and COOH termini of APP and tau protein for 12 h at 4°C. An anti-βAP antibody was raised against carrier-free β1-28. Anti-NH_2 and COOH termini of APP antibodies were raised against the NH_2-terminus 18 residues (amino acids 45–62) and COOH terminus 30 residues (amino acids 666–695) of putative APP, respectively [29]. The numbering follows APP 695. An anti-tau protein antibody was raised against human tau protein [16]. Sections were subsequently incubated for 1 h at 37°C in 1:100 diluted goat biotinylated anti-rabbit IgG (H+L) (Vector) and then incubated in streptavidin-peroxidase (Nichirei) or 1:200 diluted streptavidin-fluorescein (Oncogene Science). Immune deposits were visualized by diaminobenzidine (DAB) or examined by fluorescence microscopy. For immunohistochemical study of ubiquitin, transverse sections were fixed in cool 100% acetone. These sections were incubated for 12 h at 4°C in 1:300 diluted mouse monoclonal anti-ubiquitin antibody (Chemicon). Sections were then incubated for 1 h at 37°C in 1:100 diluted anti-mouse Ig horseradish peroxidase-linked $F(ab')_2$ fragment (Amersham). Deposits were visualized with DAB. For fluorescence microscopy, the secondary antibody was 1:300 diluted FITC-conjugated goat $F(ab')_2$ anti-mouse IgG+IgM (Tago), and the sections were incubated for 45 min at 37°C. They were examined by fluorescence microscopy. In the immunohistochemical staining, negative controls were performed by omission of the first antibodies.

Results

Histochemical and immunohistochemical findings in DMRV, IBM and controls are summarized in Table 1. In all muscle biopsies from patients with DMRV, there was notable variation in both type 1 and 2 fibers. All muscle specimens contained at least ten muscle fibers with rimmed vacuoles in a low-power field (magnification 20), which were predominantly seen in small atrophic fibers occasionally occurring in small groups. None had inflammatory cell infiltration. Except for two muscle biopsies, which had a few necrotic fibers, no apparent necrotic and regenerating process was found. No fiber-type grouping or group atrophy suggesting denervation and reinnervation was noted. On electron microscopy five of seven muscles examined contained "tubulofilamentous inclusions" in the nuclei. The filaments measured 15–20 nm in external diameter. The inclusions were similar to those seen in IBM.

In addition to the interstitial mononuclear inflammatory cell infiltration, the rimmed vacuoles were seen in all four muscle biopsies from patients with IBM. All had the characteristic tubulofilamentous inclusions in their nuclei on electron microscopy.

Of the 11 muscle biopsies from patients with DMRV (Fig. 1B, D) 8 had occasional Congophilic amyloid material in vacuolated fibers and this was also seen in 3 of 4 patients with IBM; however, vacuolated fibers did not invariably contain Congophilic material. Hyaline eosinophilic material seen in the cytoplasm of vacuolated fibers and/or degenerative fibers, which were similar to cytoplasmic/spheroid bodies seen in H&E- and modified Gomori trichrome-stained sections, also demonstrated Congo red positivity. Of the DMRV biopsies 9 were positive for βAP (Fig. 1E), 9 for the NH_2 terminus of APP (Fig. 1G), 10 for the COOH terminus of APP (Fig. 1H), 10

Table 1 Congophilic amyloid material and immunoreactive deposits of βAP, APP, ubiquitin and tau protein (*DMRV* distal myopathy with rimmed vacuole formation, *IBM* inclusion body myositis, *M* male, *F* female, *βAP* β-amyloid protein, *APPN* NH_2 terminus of β-amyloid protein precursor, *APPC* COOH terminus of β-amyloid protein precursor, + positive, ± equivocal, – negative)

Diagnoses		Age	Sex	Congo red	βAP	APPN	APPC	Ubi-quitin	Tau protein
DMRV	1	25	F	–	±	+	+	+	+
	2	28	F	+	+	+	+	+	+
	3	37	F	+	+	+	+	+	+
	4	35	M	+	+	+	+	+	+
	5	26	M	+	+	+	+	+	+
	6	67	M	+	±	±	±	+	±
	7	26	M	–	+	+	+	+	+
	8	33	M	+	+	+	+	+	+
	9	42	F	+	+	+	+	±	+
	10	60	M	+	+	+	+	+	+
	11	29	F	–	+	±	+	+	+
IBM	1	53	F	–	+	+	+	+	+
	2	61	M	+	+	+	+	+	+
	3	59	M	+	+	+	+	+	+
	4	51	M	+	+	+	+	+	+
Controls (1–10)		32–60		–	–	–	–	–	–

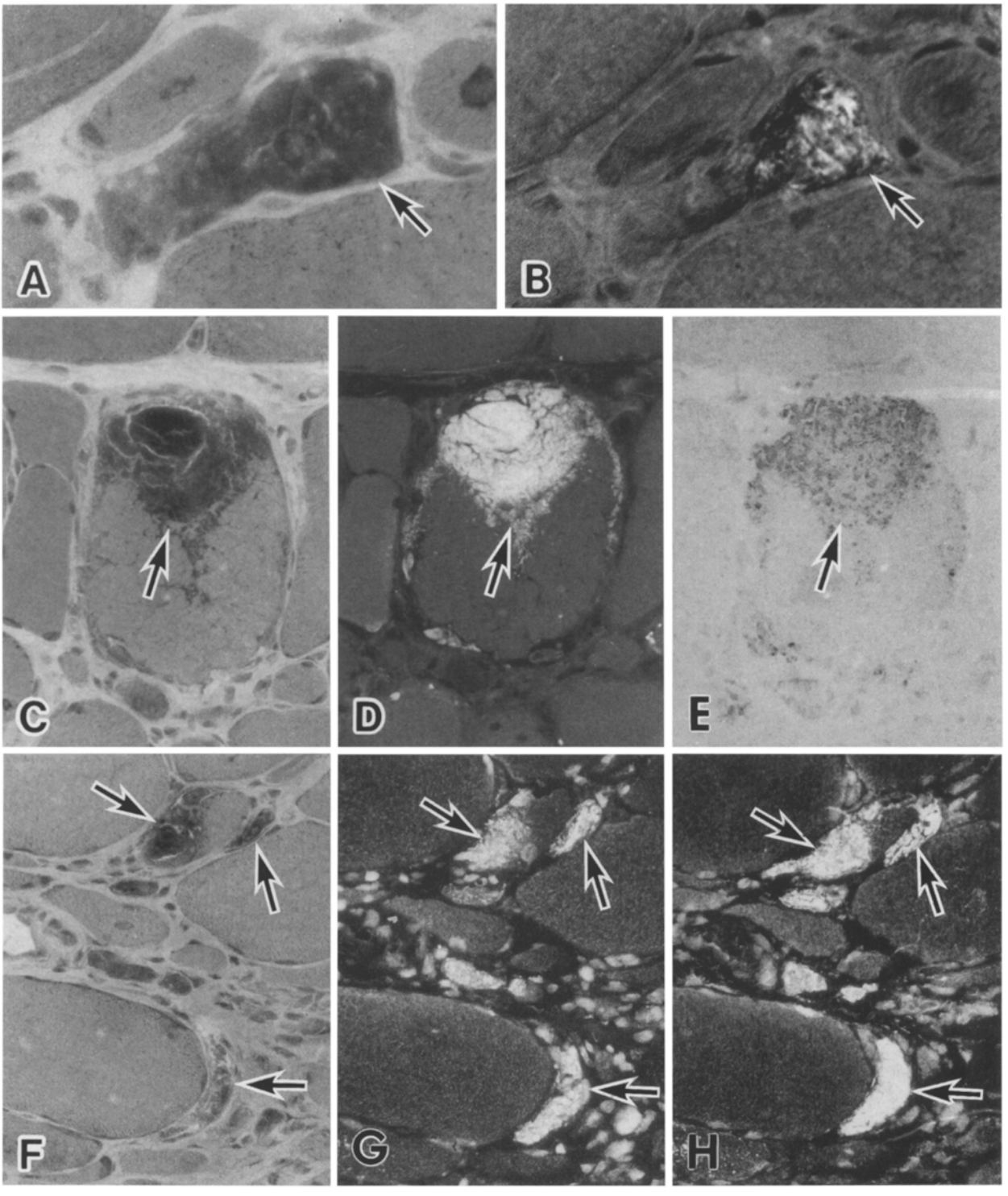

Fig. 1 The degenerative fibers with rimmed vacuoles (*arrows* in **A, C**) contain Congophilic amyloid material (**B, D**) and are stained positively with an anti-βAP antibody (**E**). They (**F**) are also immunoreactive to NH_2 (**G**) and COOH (**H**) termini of APP. Biopsy of a 42-year-old female with DMRV (patient 9). **A, C, F** Modified Gomori trichrome; **B** Congo red stain under polarized light; **D** Congo red stain under fluorescence microscopy; **E, G, H** immunohistochemistry with antibodies against βAP, and NH_2 and COOH termini of APP antibodies, respectively. **A, B** (serial sections) 650; **C–E** (serial sections) 270; **F–H** (serial sections) 290

for ubiquitin (Fig. 2B) and 10 for tau protein (Fig. 2D). The incidence of vacuolated muscle fibers with positive immunoreactive deposits against βAP, both termini of APP, ubiquitin and tau protein comprised 80%, 50–80%, 80% and 40–80%, respectively. Vacuolated and/or degenerating fibers with cytoplasmic/spheroid body-like inclusions tended to have strong immunoreactivities against both termini of APP. Immunoreactivity against ubiquitin was found in muscle biopsies with an extensive rimmed

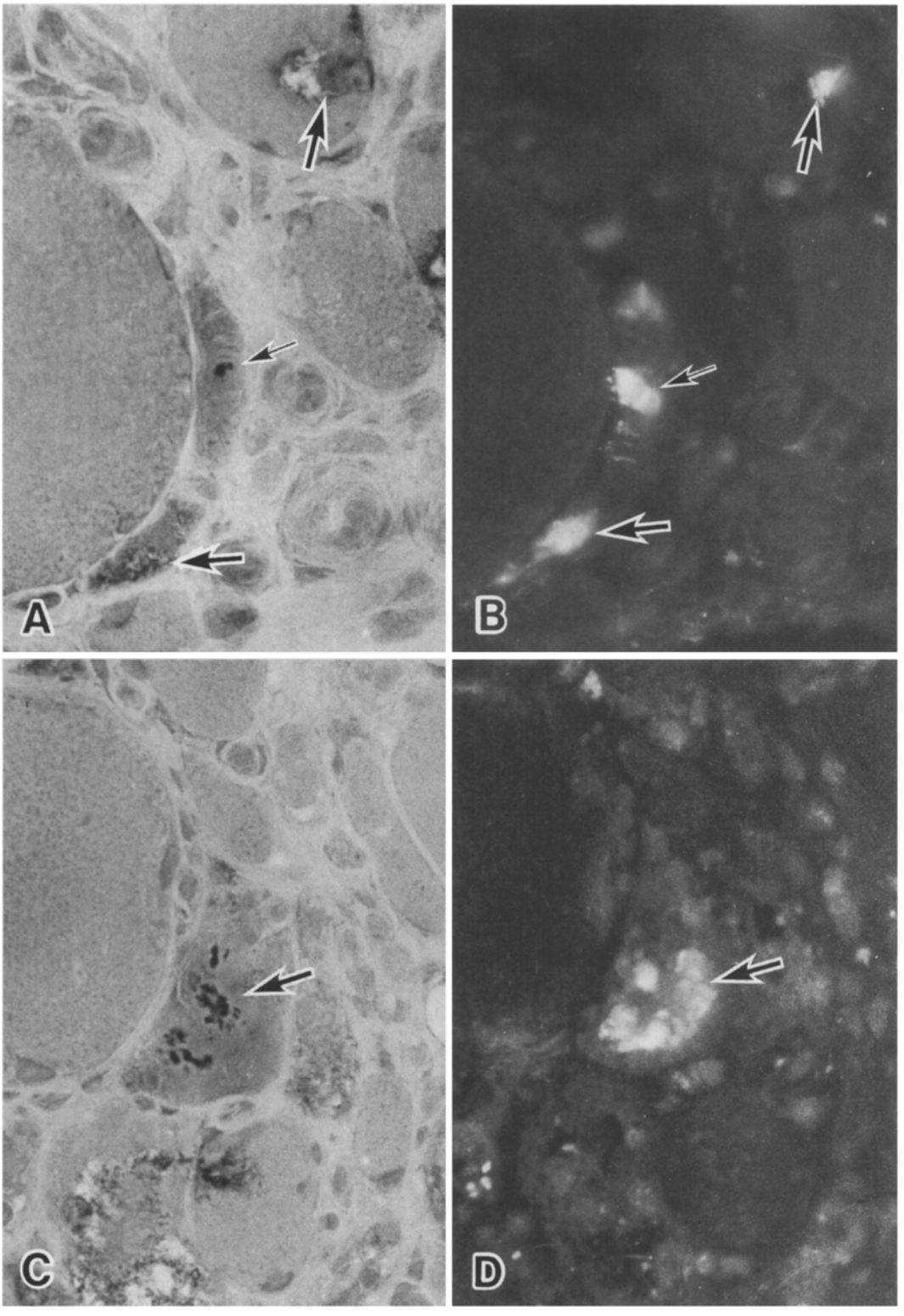

Fig. 2 Immunoreactive deposits of anti-ubiquitin antibody are seen in the rimmed vacuoles (*arrows*), especially around a cytoplasmic/spheroid body-like inclusion (*small arrow*) (**A, B**). Note strong immunoreactive deposits of anti-tau protein antibody around cytoplasmic/spheroid body-like inclusions (*arrows*) in the rimmed vacuoles (**C, D**). Biopsy of a 26-year-old male with DMRV (patient 5). **A, C** Modified Gomori trichrome; **B, D** immunohistochemistry with anti-ubiquitin and tau protein antibodies, respectively. **A, B** (serial sections) 630; **C, D** (serial sections) 600

vacuole formation. Immunoreactivity with the anti-tau protein antibody was often observed around cytoplasmic/spheroid body-like inclusions. In contrast, all four of the muscle biopsies from IBM showed positive immunoreactivities against these proteins, although the incidence of vacuolated muscle fibers with positive immunoreactive deposits against these proteins was similar to that of DMRV. All ten control muscle biopsies were negative on Congo red and immunohistochemical staining.

Discussion

DMRV is a distinct clinical entity inherited through an autosomal recessive trait with female preponderance [25, 26, 28]. The initial and preferential peroneal muscle involvement appears in the second to third decade of life in most patients. The disease is progressive, usually leading to a nonambulant state within 10 years after the onset of the disease [28]. Muscle pathology is characterized by rimmed vacuole formation without apparent muscle fiber necrosis [25, 26]. IBM is a nonhereditary disease with male predominance, showing mostly proximal muscle weakness after the age of 50 years. Pathological findings of IBM are characterized by inflammatory response, muscle fibers with rimmed vacuoles and cytoplasmic and intranuclear tubulofilamentous inclusions. Similar filamentous inclusions have been identified in both nuclei and cytoplasm in muscle biopsies from DMRV and Welander-type distal myopathy [9, 22, 25]. Welander-type distal myopathy, characterized by late adult onset and autosomal dominant inheritance, also has rimmed vacuoles in muscles. DMRV and Welander-type distal myopathy, therefore, are not distinguishable from IBM on the basis of pathological findings, except for the absence of inflammatory cell infiltration and the mode of inheritance [19, 27]. Hereditary inclusion body myopathy, as proposed by Askanas et al. [1, 4, 5], may be a group of heterogeneous disorders because rimmed vacuole formation with nuclear inclusions itself is not a disease-specific finding but is seen in various hereditary and sporadic diseases including Welander-type distal myopathy [9], Marinesco-Sjögren syndrome and other disorders [11, 27].

Mendell et al. [23] reported that atrophic fibers with rimmed vacuoles in IBM contained Congo red-positive amyloid, but that those in other neuromuscular diseases did not, concluding that this finding may be specific for IBM. Thereafter, Askanas et al. [1–5, 7, 8] reported abnormal accumulations of ubiquitin, βAP, APP, apolipoprotein E and tau protein in vacuolated muscle fibers of IBM and hereditary inclusion body myopathy. A similar abnormal accumulation of ubiquitin in vacuolated fibers was recognized in oculopharyngeal muscular dystrophy [18]. Since no Congophilic amyloid material was found in muscle biopsies of hereditary inclusion body myopathy, Askanas et al. [1] speculated that hereditary inclusion body myopathy was an "early" stage of IBM.

We performed the present study to ascertain whether the histochemical and immunohistochemical examinations can differentiate DMRV from IBM and hereditary inclusion body myopathy. Our results indicate that the degenerative process resulting in rimmed vacuole formation in DMRV is identical to that in IBM. Since Congophilic amyloid material is found in patients with DMRV, hereditary inclusion body myopathy, defined as "early" stage of IBM [1], is different from DMRV.

Since the rimmed vacuole has been shown to have a high content of lysosomal acid phosphatase [25], cysteinine protease [17] and membranous autophagic vacuoles by electron microscopy, there is little doubt that increased lysosomal activities are involved in the rimmed vacuole formation. However, it remains unknown whether increased lysosomal enzyme activity is the primary abnormality resulting in myofibrillar degeneration or whether it is a secondarily induced phenomenon to scavenge degenerative myofibrillar components. Nevertheless, an abnormal lysosomal function may play a role in generating βAP and amyloid deposition as proposed in Alzheimer's disease, because it is widely believed that βAP is generated through an endosomal-lysosomal pathway [13–15]. As Askanas and co-workers have emphasized in their many reports [1–8], if we clarify the pathomechanism of degenerative process involved in the rimmed vacuole formation in the "peripheral" muscle tissue, the results may provide some insight into the processes involved in amyloid-plaque formation in Alzheimer's disease.

Acknowledgement The authors wish to thank Dr. Hideo Sugita (President of the National Center of Neurology and Psychiatry, Japan) for his kind suggestions and advice on this work.

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