Chapter 7

Autophagy in GNE Myopathy

Anna Cho and Satoru Noguchi

Additional information is available at the end of the chapter

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1. Introduction

Muscle diseases represent specific muscle pathology. The characteristic features as hallmarks of diseases have been historically used to diagnose the patients. The “Rimmed vacuole (RV)” (Figures 1) is one of such characteristic features in certain groups of the diseases. This structure consists of the space (vacuole) and purple granules (rim) within myofibers, while the space is sometimes occupied with cytosolic contents indicating that the space is artificially produced during the staining process and the rims have the nature of this pathological hallmark. Ultrastructurally, as discussed later, many autophagic vacuoles and multi-lamellar bodies are observed in RVs.

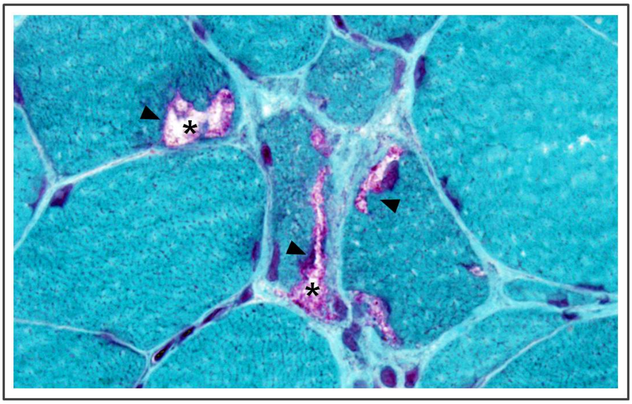

Figure 1. Rimmed vacuoles in a modified Gomori trichrome section. The purple granules (arrow heads) are surrounding vacuoles (asterisks).

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Skeletal muscle represents 40~50% of the human body and is one of the most important sites for the control of metabolism. During catabolic conditions, muscle proteins are mobilized to provide alternative energy substrates for the other organs. The RV formation indicates dysfunction of autophagy and breakdown of energy homeostasis in diseased skeletal muscles. In addition, it also suggests the importance of autophagy in muscle functions. There is a group of muscle disease, generally referred to as autophagic vacuolar myopathies (AVM), which are characterized by the accumulation of autophagic vacuoles on skeletal muscle pathology.

In this review, we will give an outline of general knowledge and classification on AVMs and overview the molecular processes underlying autophagic vacuoles formation in rimmed vacuolar myopathies on the basis of our experimental evidences regarding GNE myopathy.

2. Autophagic vacuolar myopathies

Dysfunctional autophagy is associated with several neurodegenerative disorders [1-3]. As for muscle disorders, these are referred to as AVMs [4]. Since the autophagosomes are not observed in normal muscle fibers, autophagic vacuoles have been often recognized as pathologic hallmarks of numerous neuromuscular disorders. Two major categories in AVMs include lysosomal myopathies and rimmed vacuolar myopathies (Table 1) [4-6]. The former are associated with a primary defect in lysosomal proteins and the two best-described and genetically diagnosable AVMs, Pompe disease and Danon disease, are classified in this group. In contrast, autophagic vacuoles in rimmed vacuolar myopathies are secondarily caused by extra-lysosomal defects and usually observed at later stages of the disease. There are various kinds of rimmed vacuolar myopathies including sporadic inclusion body myositis (sIBM) and myofibrillar myopathies and most of them are clinically and etiologically heterogeneous disorders.

Disease	Causative Genes
Lysosomal Myopathy	
Acid maltase deficiency (Pompe disease)	*GAA*
Danon disease	*LAMP2*
X-linked myopathy with excessive autophagy (XMEA)	(identified)
Myopathy with rimmed vacuoles (RVs)	
Inclusion body myositis (sIBM)	?
Myofibrillar myopathy	*DES CRYAB MYOT ZASP etc*
GNE myopathy	*GNE*
Inclusion body myopathy, Paget's disease of bone, and frontotemporal dementia (IBMPFD)	*VCP*
Other myopathies often showing RVs	
Oculopharyngeal muscular dystrophy (OPMD)	*PABPN1*
Marinesco-Sjögren syndrome	*SIL1*
Myotonic dystrophy	*DMPK*

Table 1. Lists of autophagic vacuolar myopathies.

2.1. Lysosomal myopathy

Two best known disorders in AVMs are associated with primary lysosomal protein defects, namely, Pompe disease [7] and Danon disease [8]. The former is caused by a deficiency of lysosomal enzymes within the vacuoles, whereas the latter is caused by a deficiency of lysosomal membrane structural protein [9].

2.1.1. Pompe disease

Pompe disease [7], also referred to as glycogen storage disease type II and acid maltase deficiency, is the best characterized lysosomal myopathy caused by a deficiency of acid α-glucosidase (GAA, also known as acid maltase). This enzyme defect results in lysosomal glycogen accumulation in multiple tissues and cell types, with skeletal and cardiac muscle cells the most seriously affected [10, 11]. The classic infantile form is a rapidly progressive disease with hypotonia, generalized muscle weakness, and hypertrophic cardiomyopathy, usually leading to death from cardiorespiratory failure or respiratory infection in the first year of life [12]. But enzyme replacement therapy with recombinant human GAA is now available, which can dramatically improve the clinical features and life expectancy of the infantile Pompe disease patients [13-15]. The late-onset type shows less progressive clinical characteristics and absence of severe cardiomyopathy; these phenotypical differences are related to residual enzyme activity [16]. The *GAA* gene is located on human chromosome 17q25.2-25.3 and more than 200 pathogenic sequence variations have been characterized up to date [17].

On muscle pathology, cytoplasmic vacuoles are so remarkable and large that these occupy most of the space in many muscle fibers (Figure 2). The vacuoles contain amorphous materials that are presumably glycogen because of the strong reactivity with periodic acid Schiff stain. Acid phosphatase staining also shows strong signals in these vacuoles, indicating high lysosomal content [6]. In terms of pathomechanism, the failure of the lysosomal degradation of glycogen leads to the accumulation of autophagic vacuoles, which may cause cellular dysfunction and abnormal cytoskeletal organization [18].

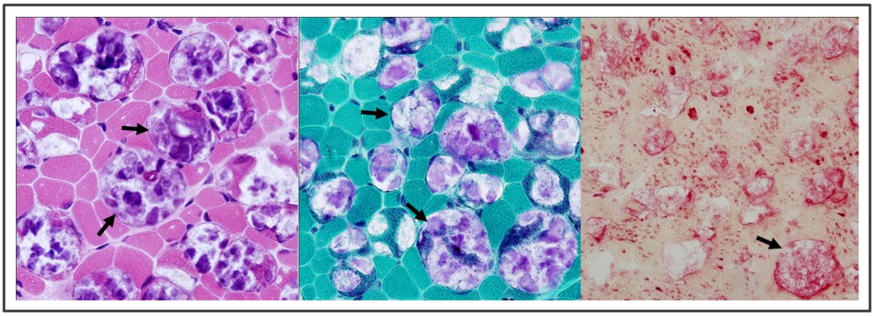

Figure 2. Pathologic findings in Pompe disease. Hematoxylin and eosin (left) and modified Gomori trichrome (middle) sections show pathognomonic vacuolar structures (arrows) in myofibers. These vacuolar structures are strongly stained by acid phosphatase (right).

2.1.2. Danon disease

Danon disease is an X-linked disorder caused by the primary deficiency of lysosome-associated membrane protein-2 (LAMP-2) [9]. Characteristic clinical features include skeletal myopathy, cardiomyopathy, and mental retardation. Male patients usually manifest the disease in their teens and die before their 30s from cardiac problems. LAMP-2 deficiency causes accumulation of autophagic vacuoles in a variety of tissues, including skeletal and cardiac muscles [5]. As LAMP-2 is required for the maturation of early autophagic vacuoles by fusion with endosomes and lysosomes, deficiency of LAMP-2 leads to a failure in the normal progression of autophagic maturation [6].

Muscle biopsy from the patients with Danon disease show scattered small basophilic granules in myofibers and lysosomal acid phosphatase activity is increased in these granules (Figure 3). Large vacuolar structures having sarcolemmal features with acetylcholine esterase activity are surrounding those lysosomal granules and these structures are known as autophagic vacuoles with sarcolemmal features (AVSF) [19]. This characteristic pathology in Danon disease (AVSF) is also seen in a number of diseases including X-linked myopathy with excessive autophagy (XMEA) [20], infantile autophagic vacuolar myopathy [21], and X-linked congenital autophagic vacuolar myopathy [22]. The list of this group of autophagic vacuolar myopathy is rapidly expanding [5] and they are expected to be related with lysosomal function because the pathologic features are quite similar to those in Danon disease.

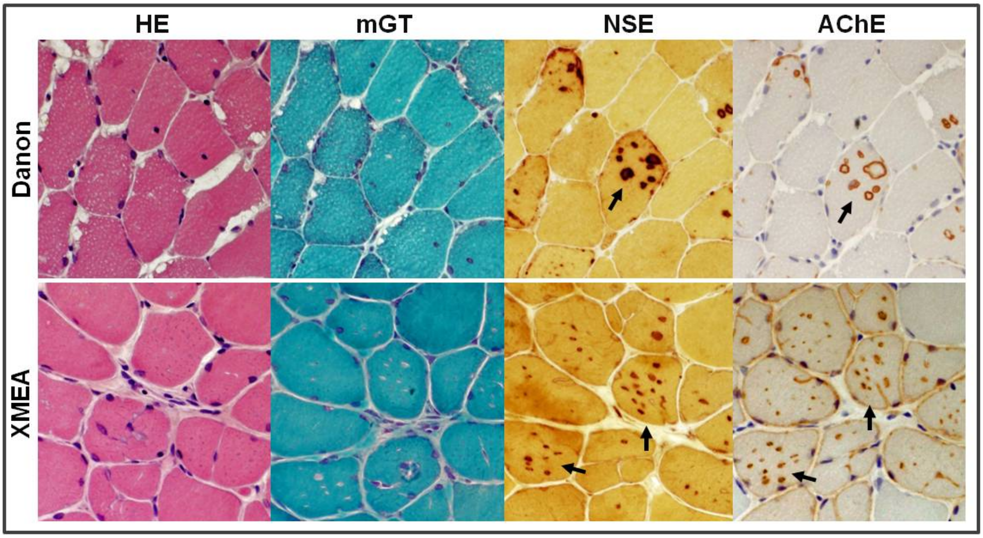

Figure 3. Pathologic findings in Danon disease and XMEA. Many AVSFs (arrows) are showing acetylcholine esterase and nonspecific esterase positivity. HE-hematoxylin and eosin; mGT-modified Gomori trichrome; NSE-nonspecific esterase; AChE-acetylcholine esterase.

2.2. Myopathy with rimmed vacuoles

Rimmed vacuolar myopathies comprise more various and heterogeneous disorders. The most common disease in this group is sIBM, which has been traditionally considered as an inflammatory myopathy. Myofibrillar myopathy, a group of chronic myopathies with a similar pathologic phenotype, is caused by several different genes. And VCP myopathy and GNE myopathy are well known single gene disorders which can be classified as hereditary inclusion body myopathies (hIBM). In addition, it is not uncommon that rimmed vacuoles are appreciated in numerous chronic myopathies which are not classically classified as rimmed vacuolar myopathies.

2.2.1. Inclusion Body Myositis (sIBM)

sIBM is the most common muscle disease in elderly patients [23-25]. Clinically, general progressive muscle weakness starts after age 50 years. The quadriceps muscle and finger flexors are usually affected early on. sIBM Patients may become unable to perform daily living activities and require assistive devices within 10 years of symptom onset. Muscle biopsy characteristically reveals rimmed vacuolar muscle fibers with endomysial T-cell inflammatory infiltrates. Although there still remains controversy whether sIBM is an autoimmune inflammatory myopathy or a primary degenerative myopathy with secondary inflammation, it is becoming more likely that abnormal myoproteostasis and muscle fiber degeneration with aging play primary pathogenic roles in this disorder [26].

Askanas and Engel [27] indicated that several phenomena observed in the degeneration of sIBM muscle fibers are similar to the neuronal degenerative processes occurring in both Alzheimer's disease and Parkinson's disease. Abnormal accumulations of various pathogenic proteins, posttranslational modifications of the accumulated proteins, abnormal protein disposal, and impaired autophagy and 26S proteasome function are common intracellular features of neurodegenerative disorders and thus suggest that sIBM is, like neurodegenerative diseases, a complex degenerative disorder caused by protein misfolding and associated with multiprotein aggregation [28].

2.2.2. Myofibrillar myopathies

RVs are often appreciated in large numbers of myofibrillar myopathies [29-31], which is a group of hereditary myopathies pathologically characterized as markedly disorganized myofibrils with cytoplasmic inclusion. Clinical symptoms of myofibrillar myopathies are very variable. The onset age rages from infancy to the eighth decade. Some patients show limb girdle muscle involvement, whereas others show distal myopathy. Cardiomyopathy is often involved and even can be seen in patients with no obvious skeletal muscle weakness. Seven disease-related genes have been identified (*DES*, *CRYAB*, *MYOT*, *ZASP*, *FLNC*, *BAG3*, and *FHL1*) up to date and all of them encode proteins closely related to Z-line. Electron microscopy findings imply that disintegration of myofibrils near Z-line causes accumulation of filamentous material and aggregation of membranous organelles and glycogen, leading to the entrapment of dislocated membranous organelles in autophagic vacuoles [31].

In the cardiomyocytes-restricted *CRYAB* over-expressed mice, autophagic activity is increased in response to protein aggregates and blunting autophagy *in vivo* dramatically worsen the disease progression [32]. Although myofibrillar myopathy includes various genetically and clinically heterogeneous disorders, accumulation of misfolded proteins is considered as a common pathological pattern and autophagy in myofibrillar myopathy is now becoming to be considered as an adaptive response.

2.2.3. Inclusion Body Myopathy, Paget's disease of the bone, and Frontotemporal Dementia (IBMPFD); Valosin-Containing Protein (VCP) myopathy

Inclusion body myopathy (IBM) with Paget's disease of bone (PDB) and frontotemporal dementia (FTD), now called IBMPFD or valosin-containing protein (VCP) myopathy, is a progressive autosomal dominant disorder caused by mutations in the *VCP* [33]. It is a rare multisystem degenerative disorder with three variably penetrated phenotypic features [34]. 90% of patients develop muscle weakness with a mean onset of 45 years of age and 50% of patients have osteolytic lesions consistent with PDB at the same mean age. About 30% patients develop a typical FTD manifested by apparent language and behavior dysfunction at fifties [35]. Other phenotypic features have been reported as well, including dilated cardiomyopathy, cataracts and sensory-motor neuropathy [36]. Muscle biopsy shows degenerating fibers with RVs and sarcoplasmic inclusions. While molecular pathogenesis in IBMPFD is unknown, the extensive accumulation of ubiquitin conjugates in affected tissues suggests impairment of protein degradation pathways in this disease. In addition, impaired maturation of ubiquitin-containing autophagosomes in cells expressing *VCP* mutants imply that defective autophagy also contributes to the pathogenesis of IBMPFD [37].

2.2.4. Other myopathies often related with rimmed vacuoles

Although they are not pathognomonic, RVs are often accompanied in various chronic myopathic conditions including Marinesco-Sjögren syndrome and oculopharyngeal muscular dystrophy (OPMD). It is interesting that these clinically and genetically different disorders are sharing a similar pathologic feature in skeletal muscles.

Marinesco-Sjögren syndrome is an autosomal recessive disorder clinically characterized by cerebellar ataxia, cataracts from infancy, mental retardation, and myopathy [38]. Loss of function mutations in *SIL1*, which encodes a nucleotide exchange factor for the Hsp70 chaperone BiP, was identified as a causative gene. Previous ultra-structural study showed that myofibrillar degeneration with autophagic phenomenon is prominent in Marinesco-Sjögren syndrome muscles [39]. In addition, it was demonstrated that increased ER stress and altered protein folding lead to neurodegeneration in *SIL1* knock-out mice [40], from which we can infer similar pathogenic process may occur in skeletal muscles of Marinesco-Sjögren syndrome.

OPDM is known to be caused by repeat expansion mutations in *PABPN1* [41]. It has recently become evident that autophagy has an important role in the pathogenesis of repeat expansion disease [42]. The role of autophagy has been extensively studied especially in the polyglutamine diseases such as Huntington's disease and spinocerebellar ataxia. Most of research evidences suggest that autophagy has up-regulated for the degeneration of misfolded proteins and usually is neuroprotective in those disorders.

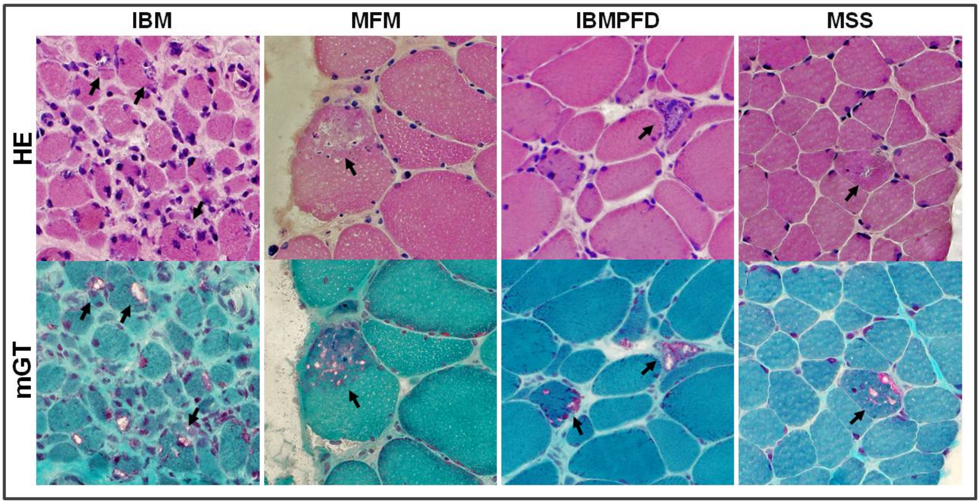

Figure 4. Pathologic findings in myopathies with rimmed vacuoles. Clinically and etiologically different disorders are showing same pathologic features (RVs; arrows) in skeletal muscles. HE-hematoxylin and eosin; mGT-modified Gomori trichrome; IBM-inclusion body myositis; MFM-myofibrillar myopathy; IBMPFD-inclusion body myopathy with Paget's disease of bone and frontotemporal dementia; MSS-Marinesco-Sjögren syndrome.

3. GNE myopathy

GNE myopathy is one of the well described rimmed vacuolar myopathies. It is an autosomal recessive myopathy originally reported in 1981 by Nonaka et al. [43, 44], and thus is also referred as distal myopathy with rimmed vacuoles (DMRV) or Nonaka myopathy. In 1984, Argov and Yarom [45] reported a similar disorder among Iranian Jews with the title of 'rimmed vacuolar myopathy sparing quadriceps'. And the term 'quadriceps sparing myopathy' and 'hereditary inclusion body myopathy (hIBM)' has also been used to refer this disease. Since these two disorders are thought to be identical and caused by GNE mutations [46], it would be better to harmonized the naming of this disease. The experts have recently designated the disease to be called "GNE myopathy".

Among the AVMs secondarily caused by extra-lysosomal defects, GNE myopathy has some advantages for the pathomechanism research. It is a single gene disorder with a homogeneous phenotype and the model mice which evidently display similar features of a human GNE myopathy have been generated [47]. Regardless of the upstream causes, autophagy in myopathies is thought to be mainly attributed to abnormal lysosomal function regarding their effects on myofiber breakdown in common and RVs appreciated in various kinds of myopathies is known to share similar histological and biochemical features. Thus, a comprehensive review of achievements and addressed issues in GNE myopathy research can broaden our understanding of this common pathomechanism of autophagy in rimmed vacuolar myopathies.

3.1. Clinical and pathologic features of GNE myopathy

Clinically, GNE myopathy is an early adult-onset progressive myopathy that affects the tibialis anterior muscle preferentially but spares quadriceps femoris muscles. The symptoms of distal limb muscle weakness start to affect the patient from the second or third decades, and most of the patients become wheelchair-bound between twenties and sixties with a median time to loss of ambulation of 17 years after disease onset [48]. Although the tibialis anterior muscle is most significantly affected, gastrocnemius, hamstrings, paraspinal, and sternocleidomastoid muscles are also involved from an early stage. Cardiac and respiratory muscles are less involved. Serum creatine kinase (CK) levels are normal to mildly elevated [49].

Muscle pathology (Figure 5) is characterized by the presence of RVs predominantly in atrophic fibers, which are occasionally aggregated and form small groups. These RVs are actually clusters of autophagic vacuoles and multi-lamellar bodies. They often contain congophilic amyloid material and deposits that are immunoreactive to β–amyloid and its precursor protein, ubiquitin, and tau protein. Ultrastructurally, the filamentous inclusions measuring 15-20 nm in diameter are seen in both cytoplasm and nucleus with the presence of autophagic vacuoles. Necrotic and regenerating fibers can be rarely seen in GNE myopathy [4, 43, 44, 49].

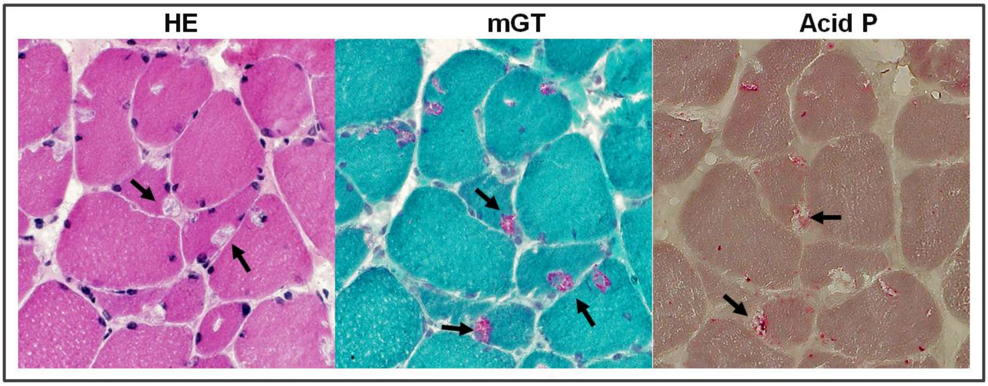

Figure 5. Pathologic findings in GNE myopathy. Rimmed vacuoles (arrows) are predominantly present in atrophic fibers. HE-hematoxylin and eosin; mGT-modified Gomori trichrome; Acid P-acid phoaphatase.

3.2. Molecular pathomechanism of GNE myopathy

GNE myopathy is caused by mutations in the gene encoding a key enzyme in sialic acid biosynthesis, UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (GNE) [50]. Previous studies have shown that predominant *GNE* mutations exist in certain populations, such as the V572L mutation in Japanese patients and the M712T mutation in Middle Eastern Jews [46, 50-52]. But it is now evident that GNE myopathy is not restricted to people of Japanese and Jewish ancestry, but they are widely distributed throughout all ethnic groups [53-56]. Recent study showed that homozygosity for V572L (the most common mutation in Japanese population) resulted in more severe phenotypes with earlier symptom onset and faster disease progression, implying the existence of genotype/phenotype correlation in GNE myopathy [48].

After the identification of *GNE* mutations, we found that GNE enzymatic activity measured *in vitro* in cells transfected with mutated *GNE* constructs is decreased. And we demonstrated that the levels of sialic acid in primary cultured cells from GNE myopathy patients are reduced and can be corrected by the addition of free sialic acid [57]. But the mechanism how the hyposialylation makes the pathognomonic pathologic change in skeletal muscle of GNE myopathy has still remained unknown. To answer the question, we developed a model mouse for GNE myopathy.

4. Animal model: A *Gne* knock out mouse expressing human *GNE* D176V mutation

Since the null mutation in *GNE* is known to be embryonically lethal [58], we adopted a different strategy to generate Gne$^{-/-}$hGNED176VTg, a mouse model for GNE myopathy [47, 59]. Our model harbored a transgene of D176V mutated human *GNE* (one of the most prevalent mutations among Japanese GNE myopathy patients) but is knocked-out of endogenous *GNE*, resulted in that only mutated GNE proteins were highly expressed and the endogenous one was disrupted. These mice exhibited marked hyposialylation in serum, muscle and other organs and reproduced similar myopathic phenotypes seen in the skeletal muscles of human GNE myopathy patients.

The muscle weakness, decreased whole muscle mass and reduced contractile power appeared in an age-related manner [60]. After 20 weeks of age, the GNE myopathy mice started to show physiologic muscle weakness, observed as impaired motor performance and reduced force generation of the skeletal muscle. This reduction of the force might be attributed to muscle atrophy, as specific twitch and tetanic forces per cross-section area are maintained at this age. The reduction in gross size of the skeletal muscle is accompanied by an increase in the number of small angular fibers. After 30 weeks of age, specific force generation in the gastrocnemius and tibialis anterior muscles was notably reduced, while myofiber size variation became more remarkable. Intracellular deposition of amyloid and other various proteins was appreciated in the gastrocnemius muscle at this age. After 40 weeks, the muscle force generation worsened, as reflected by increased twitch/tetanic ratio, which might be due to the appearance of the characteristic RV and accumulation of autophagic vacuoles [61]. With these results, the GNE$^{-/-}$hGNED176VTg mouse is the only existing pathogenic model for GNE myopathy up to date.

Muscle pathology of GNE myopathy mice reveals RVs after 40 weeks of age (Figure 6). Intense acid phosphatase staining and expression of lysosomal-associated membrane proteins (LAMPs) and LC3 imply that autophagic process is activated in skeletal muscles of the model mice [47]. Inclusion bodies are also appreciated with expression of various protein markers.

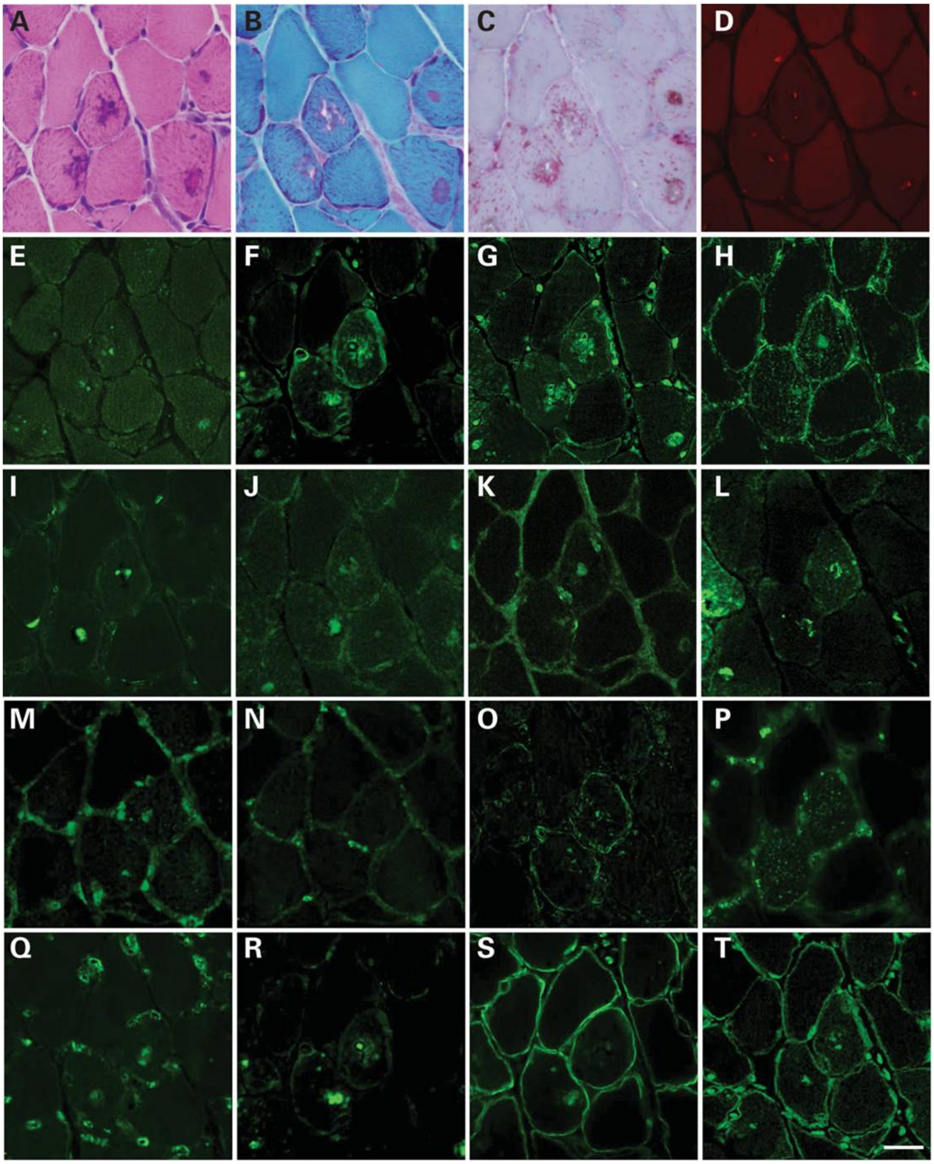

Figure 6. Pathologic findings in the GNE myopathy model mouse. (A) Hematoxylin and eosin sections showing fibers with RVs and cytoplasmic inclusions. (B) modified Gomori trichrome; (C) Acid phosphatase; (D) Congo red. Immunoreactivity to lysosomal proteins confirm the presence of autophagy in fibers with RVs. (E) LAMP-1; (F) LAMP-2; (G) LC3. Intracellular deposition of amyloid is seen in vacuolated or nonvacuolated fibers. (H) BACE2; (I) AβPP; (J) β-amyloid 1-42; (K) β -amyloid 1-40; (L) β -amyloid oligomeric. Neurofilament deposition is observed in the myofibers. (M) SM-31; (N) SM-310. (O) phosphorylated tau; (P) ubiquitin; (Q) Grp94. Sarcolemmal proteins are deposited within the vicinity of RVs. (R) α-dystroglycan (S) β-dystroglycan (T) α-sarcoglycan. Bar-20μm. (Reproduced from [47])

4.1. Autophagy in a mouse model of GNE myopathy

The characteristic RVs are observed after 40 weeks in the GNE myopathy model mouse. Like human muscle pathologic findings, these vacuoles have high acid phosphatase activity and strongly stained by various lysosomal antibodies (Figure 6) [47]. Ultrastructurally, the RVs contain multi-lamellar bodies, electron-dense bodies, and heterogeneous cytoplasmic debris which are surrounded by double membranes, indicating these are autophagic vacuoles (Figure 7). In the near areas, several vacuoles have a single limiting membrane and some cellular debris have no membrane, indicating degradated or ruptured vacuoles. Interestingly, filamentous or granular deposits considered as amyloid often appear with the autophagic vacuoles. And these probable amyloid deposits are also observed in the normal areas, which may suggest that the deposition of protein precede the accumulation of autophagic vacuoles [61].

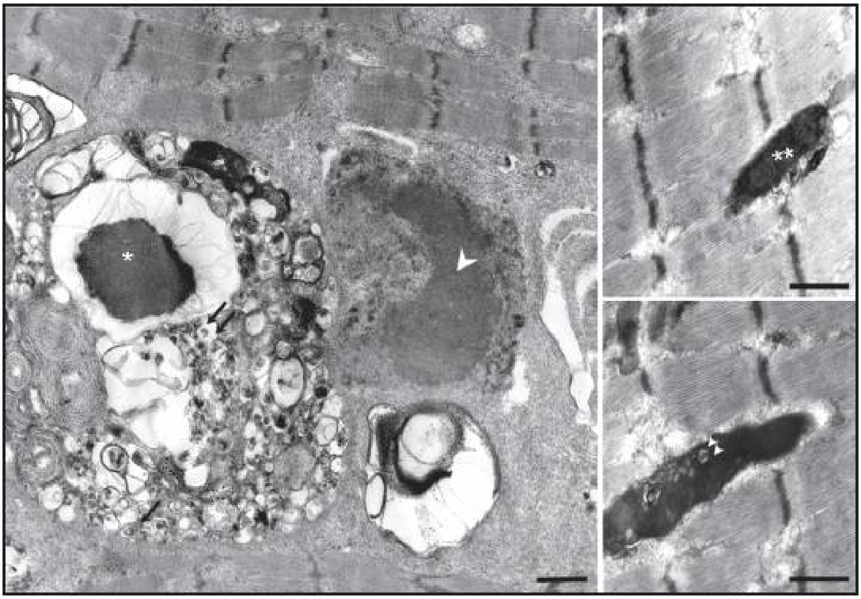

Figure 7. Electron microscopy findings in the GNE myopathy mouse model. Various cellular debris are enclosed by nacent (arrows) and degenerative (double arrows) autophagic vacuoles. Large osmiophilic doposits (asterisk) can be also seen. Dense ovoid bodies (double asterisk) are seen with autophagic vacuoles (double arrowheads) suggesting that these deposits predate RVs formation. Bars-1μm. (Reproduced from [61])

5. Prophylactic treatment with sialic acid metabolites in the GNE myopathy model mice

A possibility of the development of therapy for GNE myopathy was demonstrated in our model mice. As the addition of sialic acid metabolites has been shown to recover overall hyposialylation in cells [57], we have challenged in administering sialic acid compounds *in vivo*. We administered 2-epimerase/N-acetylmannosamine (ManNAc) to the GNE myopathy model mice from the preclinical age (5~6 weeks) continuously until the mice reached the age when all myopathic symptoms appear (54~57 weeks). ManNAc was added to drinking water and given in three doses: 20mg (low dose), 200mg (medium dose), and 2000mg (high dose)/kg body weight of mice in a day. During the treatment period, survival rate was remarkably improved as compared with control-treated mice at all three doses (Figure 8). At the end of the treatment, the phenotypes of the mice were evaluated and compared with placebo group and non-affected littermates. At all doses, motor performance and physiological contractile properties of skeletal muscles were remarkably improved. Sialic acid levels in the blood and tissues were elevated, and more importantly, the levels of sialic acid in the muscle were recovered to an almost normal level after treatment, providing evidence that prophylactic oral administration of ManNAc to the the model mice was notably effective. Then we also examined the effect of oral *N*-acetylneuraminic acid (NeuAc) and sialyllactose together with minimum dose of ManNAc (20 mg/kg bodyweight/day) on GNE myopathy mice starting at the preclinical age of 10~20 weeks. Treatment was also continued up to 54~57 weeks of age and similar beneficial effects on motor performance and force generation of skeletal muscles were obtained [62].

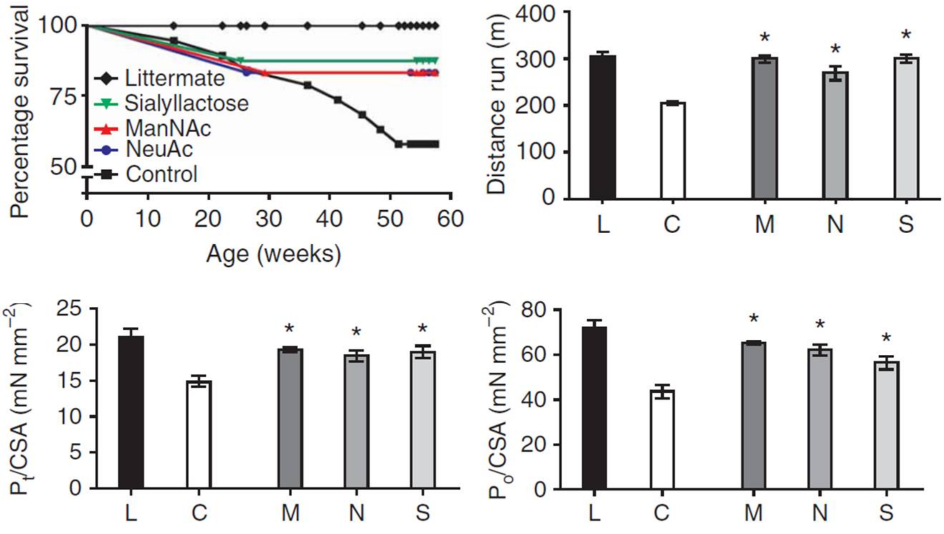

Figure 8. Favorable effects of sialic acid metabolites administration in GNE myopathy model mice. Survival curves (left upper), treadmill performance test (right upper), and contractile properties of specific isometric force (P_t/CSA; left lower) and specific tetanic force (P_0/CSA; right lower). (Reproduced from [62])

Sialic acid metabolites administration also led to a marked change in the muscle pathology of GNE myopathy mice (Figure 9) [62]. Although all mice in the control-treated group showed

RVs in the gastrocnemius muscles, only a few in the treatment groups showed RVs. As RVs are autophagic in nature, we checked for acid phosphatase staining and found decreased staining. The expression of LC3 and Lamp2, which are markers for autophagosomal structures, were not observed in the muscle sections of ManNAc treated mice, except for one mouse that had few RVs in the muscle. The amounts of LC3-I and LC3-II, used as an index to analyze autophagic induction in tissues, were lower after treatment. Treatment also increased muscle cross-sectional area (CSA) and diminished congophilic, amyloid-positive and tau-positive inclusions.

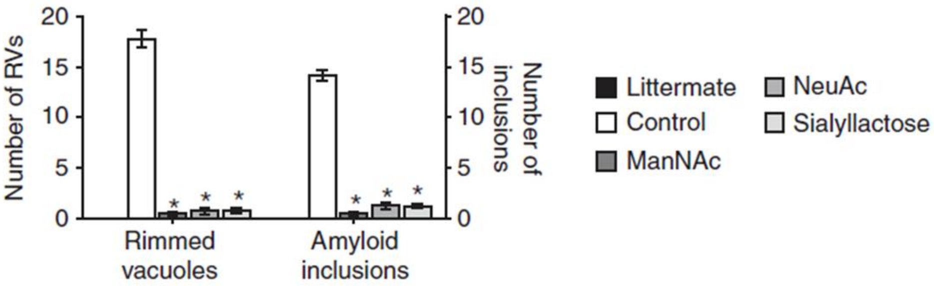

Figure 9. Pathologic improvement after treatment with sialic acid metabolites in GNE myopathy model mice. (Reproduced from [62])

Our successful prophylactic treatment results on the model mice supports the current concept that hyposialylation is one of main factors contributing the pathogenesis of GNE myopathy. This concept suggests that GNE myopathy is a potentially treatable disease and A phase I clinical trial for human patients using oral sialic acid therapy is recently underway in Japan.

6. Future issue 1 — Hypothesized pathway from hyposialylation to RVs formation

Although it has been already demonstrated that hyposialylation is a key factor in the pathomechanism of GNE myopathy and sialic acid metabolites administration can prevent the development of myopathic phenotype in GNE myopathy model mouse, there still remains an unexplained link between hyposialylation due to GNE mutations and the pathognomonic findings in the muscle. However, since the the model mice exhibit hyposialylation and intracellular amyloid deposition before the characteristic RVs appear, we can appreciate that the dysfunctional autophagy is a downstream phenomenon to hyposialylation and amyloid deposition in GNE myopathy.

In normal macroautophgy process, cytoplasm and organelles are enclosed by an isolated membrane (phagophore) to form an autophagosome. The outer membrane of the autophagosome fuses with the lysosome, and the internal material is degraded in the Autolysosome [63]. However, in hyposialylated condition, the autophagy does not progress normally. As hyposialylation can lead to abnormal protein configuration or misfolding, an excessive amount of misfolded glycoproteins which were not degraded in the ER may cause abnormal autophagy in GNE myopathy (Figure 10). Hyposialylation may also lead to abnormal protein processing which can induce abnormal protein deposits in cytoplasm. In addition, there are several reports that suggested oxidative stress is involved in the upstream pathways to amyloid deposition and/or RVs formation. One previous report showed that autophagic vacuoles were associated to be a site of amyloidogenic amyloid precursor protein processing and intra-lysosomal amyloid-β accumulation was induced by oxidative stress [64]. And another report demonstrated that reactive oxygen species may contribute to the formation of autophagosomes [65]. An experimental result implying a biologic function of sialic acid as an oxygen radical scavenger suggests hyposialylation can directly contribute to the increase of oxidative stress and support the above concept [66].

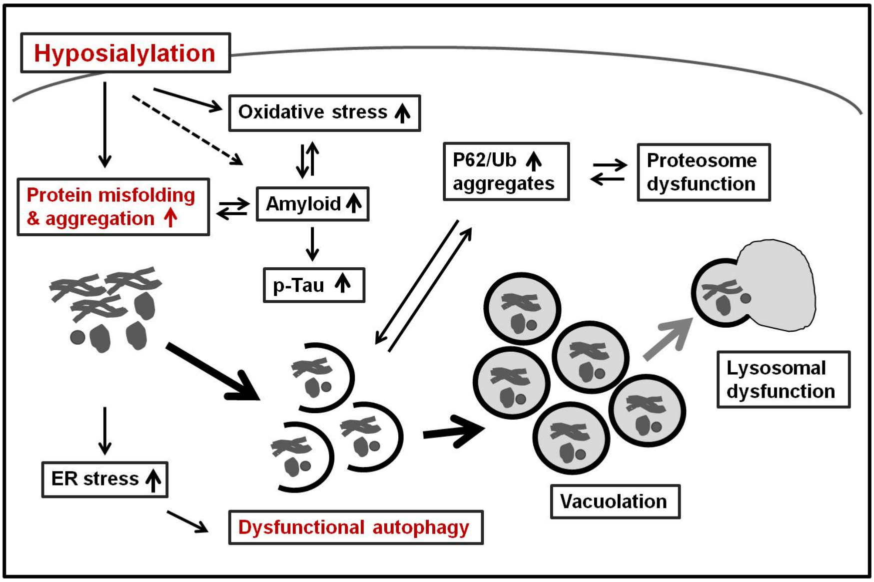

Figure 10. Hypothesized mechanism of dysfunctional autophagy in GNE myopathy.

7. Future issue 2 — The common molecular processes underlying autophagic vacuoles formation in rimmed vacuolar myopathies

Regarding the pathomechanism of rimmed vacuolar myopathies, it is interesting to figure out how various kinds of myopathies from different etiologies can share the similar pathology and undergo similar pathogenic process. One of the most impressive finding is that the accumulation of misfolded proteins and subsequent activation of autophagy are observed in all kinds of rimmed vacuolar myopathies commonly and constantly, which bring us that intra-myofiber accumulation of conformationally modified proteins plays a primary pathologic role in these disorders. However, up-regulation of autophagy alone may not be enough to form RVs if the pathways are operating properly. The primary role of autophagy is to protect cells under stress conditions and it is widely accepted that in most of neurodegenerative diseases, activation of autophagic process is an adaptive response against disease-related stress conditions. The fact that not all myofibrillar myopathy muscles show RVs suggests that the other pathways are necessary to complete RV formation.

Dysfunctional autophagy is another important common feature in rimmed vacuolar myopathies. It was already demonstrated that autophagy is impaired in sIBM, IBMPFD, GNE myopathy, and Marinesco-Sjögren syndrome [26, 37, 40, 61]. Regardless of upstream process, autophagosomes are proliferated and enlarged with lysosomal dysfunction and macroautophagy disregulation, and it might be contribute or worsen the abnormal accumulation of various proteins such as amyloids, p-tau, α-synuclein, and p62. This two major common process, up-regulated and dysfunctional autophagy, possibly develop characteristic RVs in skeletal muscle pathology.

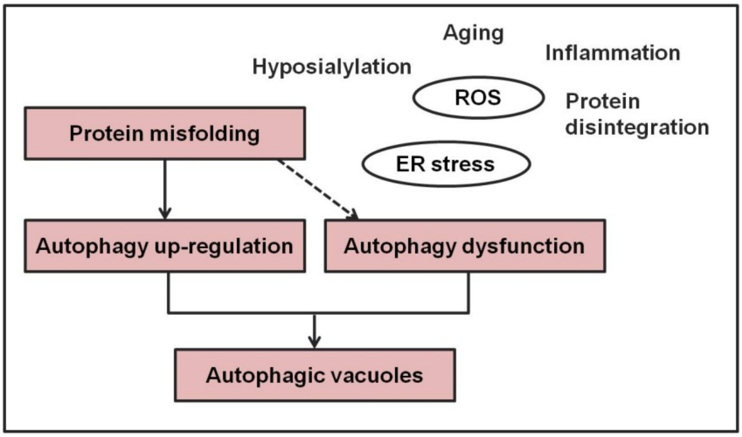

Figure 11. Hypothesized common mechanism of rimmed vacuoles formation in various AVMs.

8. Conclusion

Herein we presented the current knowledge on AVMs and recent approaches to the pathogenesis of rimmed vacuolar myopathies. With the model mice, we proved that hyposialylation is a key factor in the pathomechanism of GNE myopathy. And we also provided evidences that prophylactic treatment with sialic acid metabolites prevents the myopathic phenotype and substantially reduced the number of RVs in the GNE myopathy mice. Since rimmed vacuolar myopathies have revealed to share common pathways regarding the autophagic vacuoles formation irrespective of heterogeneous clinical phenotype and etiology, our experimental achievement can broaden the general understanding on the common pathomechanism of AVMs.

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Author details

Anna Cho and Satoru Noguchi*

Department of Neuromuscular Research, National Institute of Neuroscience, NCNP, Tokyo, Japan

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