Autophagic Vacuolar Myopathies

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Hereditary myopathies characterized by the development of autophagic vacuoles can be categorized into three groups: rimmed vacuolar myopathies, acid maltase deficiency (glycogen storage disease type II), and myopathies characterized by the autophagic vacuoles with unique vacuolar membranes. Rimmed vacuolar myopathies are most likely secondary lysosomal myopathies because all of the identified causative genes encode extralysosomal proteins. Deficiency of acid maltase, a lysosomal enzyme, has been well characterized clinically, pathologically, biochemically, and genetically, and may become treatable in the near future. The diseases in the last category are relatively rare, but appear to be genetically heterogeneous and the list of these diseases is expanding. Danon disease, the best-characterized disorder in this group, is caused by primary deficiency of a lysosomal membrane protein, LAMP-2. Therefore, diseases in this category are expected to be primary lysosomal disease.

Introduction

One of three pathways for degradation of cellular components in lysosomes, autophagy is probably important for all cells to eliminate waste material [1]. Morphologically, lysosomes or autophagic vacuoles are unremarkable in normal muscle. Nevertheless, in certain muscle diseases, the lysosomal system becomes prominent, indicating that autophagy is essential for myocytes.

No discernible attempt has been made to categorize human hereditary muscle diseases in terms of lysosomal abnormalities. However, the identification of a growing number of myopathies associated with autophagic vacuoles is clearly leading to the establishment of a new category of muscle diseases. In this review, I tentatively define the autophagic vacuolar myopathy as hereditary myopathies morphologically characterized by the presence of autophagic vacuoles and classify it into three groups: rimmed vacuolar myopathies, acid maltase deficiency, and autophagic vacuolar myopathies with unique vacuolar membranes.

Rimmed Vacuolar Myopathies

In muscle pathology, one of the most frequently encountered lysosomal abnormalities is rimmed vacuoles. Rimmed vacuoles are typically detected as small vacuoles lined by many red granules (the "rim") on modified Gomori trichrome staining. These vacuoles, however, are not true holes in the muscle fiber, but rather artifacts produced during the staining procedure. Ultrastructurally, rimmed vacuoles are clusters of autophagic vacuoles and myeloid bodies. These autophagic vacuoles probably detach easily from glass slides and move to the nearby myofibrils during the staining procedure. The regions where autophagic vacuoles had been clustered become empty (vacuoles), and the surrounding areas are decorated by granular autophagic vacuoles (rims).

There are a number of hereditary muscle diseases pathologically characterized by the presence of rimmed vacuoles (Table 1). Interestingly, none of the genes responsible for the diseases in this category encode lysosomal proteins; they all encode extralysosomal proteins such as distal myopathy with rimmed vacuoles (DMRV) (Fig. 1) and hereditary inclusion body myopathies (HIBM).

Distal myopathy with rimmed vacuoles was originally reported in 1981 by Nonaka *et al*. [2] as a new type of myopathy characterized by preferential involvement of tibialis anterior muscle and rimmed vacuoles in muscle pathology. The disease has also been called Nonaka myopathy. Three years later, Argov and Yarom [3] reported a disease similar to DMRV under the name of rimmed vacuole myopathy sparing the quadriceps. The latter disease is now widely known as HIBM. Both diseases are clinically characterized by an autosomal recessive mode of inheritance, onset in adolescence or adulthood, and preferential involvement of tibialis anterior muscle.

Distal myopathy with rimmed vacuoles and HIBM have long been suspected to be the same disease because the clinicopathologic features are essentially the same and both diseases map to the same chromosomal locus [4,5]. Recently, HIBM was shown to be associated with mutations in the gene *GNE*, which encodes a single protein with two enzyme activities [6••]. More recently, DMRV was also shown to be associated with *GNE* gene mutations [7]. Therefore, DMRV and HIBM are allelic and most likely are the same disease.

The protein encoded by the *GNE* gene catalyzes the rate-limiting step in the sialic acid biosynthetic pathway. The epimerase activity is reduced in lymphocytes from DMRV patients regardless of the site of the mutation,

Table 1. Hereditary myopathies with rimmed vacuoles whose genes have been identified

Disease	AD/AR	Locus	Gene product
Hereditary inclusion body myopathy	AR	9p1-q1	UDP-GlcNAc 2-epimerase/Man NAc kinase
Distal myopathy with rimmed vacuoles	AR	9p1-q1	UDP-GlcNAc 2-epimerase/Man NAc kinase
Limb-girdle muscular dystrophy 2G	AR	17q12	Telethonin
Inclusion body myopathy 3	AD	17p13.1	Myosin heavy chain IIa
Limb-girdle muscular dystrophy 1A	AD	5q31	Myotilin
Oculopharyngeal muscular dystrophy	AD	14q11.2-q13	Poly (A) binding protein 2
Desmin myopathy	AD	2q35	Desmin
Desmin-related myopathy	AD	11q22.3-q23.1	αB-crystalin
Tibial muscular dystrophy (Udd myopathy)	AD	2q24.3	Titin/connectin

AD—autosomal dominant; AR—autosomal recessive.

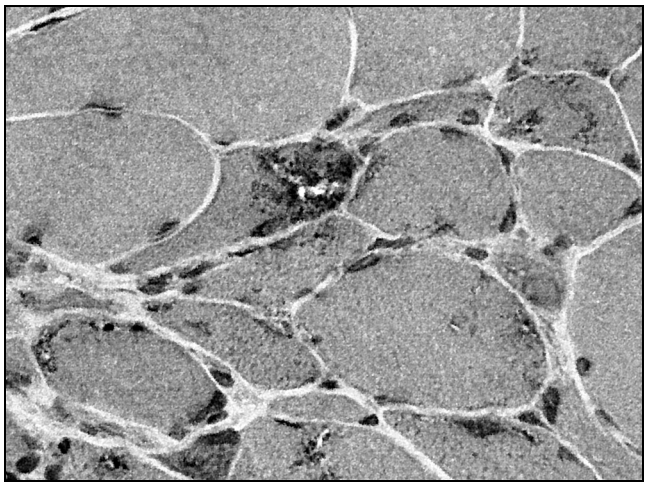

Figure 1. Rimmed vacuoles seen in distal myopathy with rimmed vacuoles using hematoxylin and eosin staining. Rimmed vacuoles are seen predominantly in atrophic fibers. This patient had a homozygous G1765C mutation in *GNE* gene.

indicating that DMRV is associated with the loss of function of the *GNE* enzyme. Therefore, the primary molecular defect resides outside of the lysosomes, indicating that rimmed vacuoles are secondarily activated lysosomes and autophagic vacuoles.

Most likely, mutations causing DMRV/HIBM and other diseases in this category result in the production of abnormal proteins or other substances that are normally degraded. One such substance is amyloid, which has been shown to be deposited in rimmed vacuolar myopathies, including HIBM, and sporadic inclusion body myositis. In fact, the over-expression of β amyloid peptide precursor (β–APP) has been shown to induce inclusion body myositis-like phenotype both in vitro [8] and in vivo [9•], although lysosomal abnormalities have not been specifically documented. The autophagic process is probably secondarily activated to degrade them. In support of this notion, other degradation systems, such as the ubiquitin-proteasome system and even apoptotic system, are also commonly activated in many of the diseases in this category, indicating that lysosomal abnormality is not the primary phenomenon. Therefore, all rimmed vacuolar myopathies are plausibly secondary lysosomal myopathies.

Acid Maltase Deficiency

The first identified primary lysosomal myopathy is glycogen storage disease type II, which is caused by the primary deficiency of acid maltase. Acid maltase is a lysosomal enzyme involved in the degradation of glycogen; therefore, undegraded glycogen is deposited both outside and within lysosomes [10]. Clinically, this disease is categorized into three groups depending on the onset of the disease: infantile, childhood, and adult-onset. The infantile form is also called Pompe disease.

Clinically the most severe, the infantile form is characterized by progressive weakness, hypotonia, and cardiac, liver, and tongue hypertrophy. The childhood form usually starts in infancy or early childhood, presenting as a predominantly proximal myopathy with preferential involvement of respiratory muscles. Heart and liver are less frequently involved than in the infantile form. Typically, patients with onset after the age of 2 years do not have cardiac manifestations. The adult-onset disease presents usually after the age of 20 years with a limb-girdle distribution of weakness and atrophy and again with preferential involvement of the respiratory muscle. It is noteworthy that the first symptom in this form can be respiratory failure.

Acid maltase deficiency (AMD) is an autosomal recessive disease, and thus both sexes are equally affected. More than 50 mutations have been reported in the gene encoding acid maltase (acid α-glucosidase), which is located on chromosome 17q25. Generally, null and deletion-type mutations cause virtually complete loss of enzyme activity and are associated with infantile AMD phenotype.

In this disease, the number and the size of vacuoles in the muscle fiber are largely proportional to clinical severity (Fig. 2). In other words, many large vacuoles are seen in the infantile form, but they are not as prominent in adult-onset form. In infantile AMD, intracytoplasmic

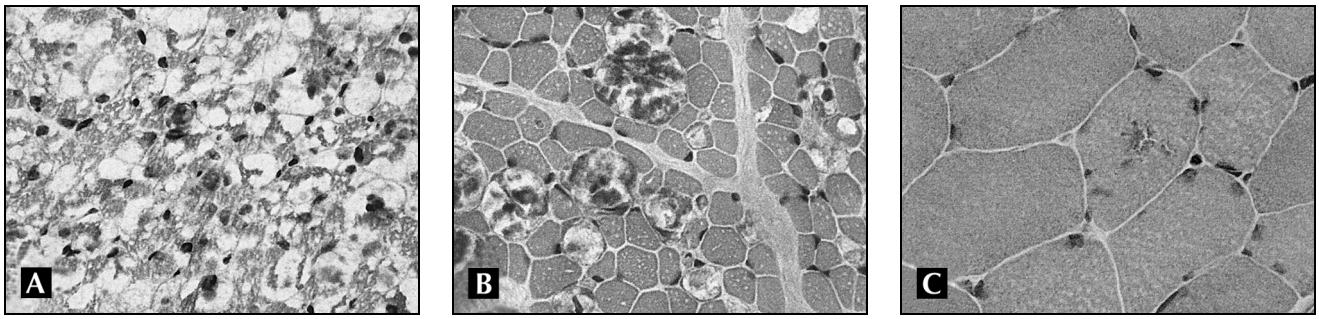

Figure 2. Muscle pathology of acid maltase deficiency (AMD) in **A,** infantile AMD; **B,** childhood AMD; and **C,** adult-onset AMD using hematoxylin and eosin staining. Note that extent of vacuolation is largely proportional to clinical severity. Infantile AMD sometimes shows "lace-like" appearance due to the extensive vacuolation, whereas in the adult-onset form vacuoles are much less prominent and resemble rimmed vacuoles.

vacuoles are so large that they occupy most of the space in many muscle fibers, often resulting in a "lace-like" appearance. These vacuoles contain amorphous materials with hematoxylin and eosin or modified Gomori trichrome stains. They are strongly highlighted with periodic acid Schiff staining, indicating that they contain glycogen. These vacuoles have high acid phosphatase activity, reflecting the lysosomal nature of the vacuoles. These vacuoles are so prominent that it is not difficult to make a histologic diagnosis of infantile AMD. In contrast, the muscle pathology of the adult-onset AMD is much milder and the vacuoles can be much less prominent. On electron microscopy, vacuoles contain cytoplasmic debris, electron dense bodies, and myelin figure, in addition to glycogen particles. Glycogen deposition is usually more prominent outside the vacuoles.

Of note, recombinant human α-glucosidase obtained from milk of transgenic rabbits has corrected the enzyme defect in knockout mice lacking acid maltase activity, which led to an ongoing clinical trial in patients.

Myopathies Characterized by Autophagic Vacuoles with Unique Vacuolar Membranes: Sarcolemmal Features

Other autophagic vacuolar myopathies include Danon disease and X-linked vacuolar myopathy with excessive autophagy. These myopathies share a unique pathologic feature: vacuolar membranes with sarcolemmal features. In addition to these two well-characterized diseases, there are likely to be more myopathies in this category, including infantile autophagic vacuolar myopathies. Although the pathomechanism underlying this unique pathologic phenomenon is a mystery, these autophagic vacuolar myopathies are likely to have genetic defects in a common pathway important for lysosomal function.

Danon disease

Danon disease was originally described as "lysosomal glycogen storage disease with normal acid maltase" by Danon *et al*. [11] in 1981 because the patients had a disease clinicopathologically similar to acid maltase deficiency but had normal enzymatic activity. However, the disease is not a glycogen storage disease because glycogen is not always increased and because the primary defect resides in lysosome-associated membrane protein-2 (LAMP-2), a lysosomal structural protein rather than a glycolytic enzyme [12••]. Consequently, Danon disease is no longer considered a lysosomal glycogen storage disease.

Danon disease is clinically characterized by the triad of hypertrophic cardiomyopathy, myopathy, and mental retardation. All probands have been male, but women do develop a milder, later-onset cardiomyopathy; therefore, the disease is transmitted in X-linked dominant mode of inheritance. In fact, the causative gene for Danon disease, *lamp-2*, is present on chromosome Xq24.

Patients have been born through normal pregnancies and deliveries. Age of onset in a study of 20 male and 18 female patients ranged from 10 months to 19 years in males and from 12 to 53 years in females [13•]. The actual onset could be earlier but remain undetected because of the insidious nature and slow progression of the disease.

All patients develop cardiomyopathy, which is the most severe and life-threatening manifestation. In male patients, cardiac symptoms, such as exertional dyspnea, begin during their teenage years. Hypertrophic cardiomyopathy and cardiac arrhythmia are common clinical signs. In a study of 38 patients with genetically confirmed Danon disease, ages at death were 19 ± 6 (mean ± SD) years for men and 40 ± 7 years for women, clearly reflecting the milder phenotype in female patients [13•].

Skeletal myopathy is usually mild and is evident in most male patients (90%), but is present in only one third of female patients. Weakness and atrophy predominantly affect neck and shoulder girdle muscles, but distal muscles can also be involved. All male patients show elevated serum creatine kinase (CK) levels, even in those without apparent muscle symptoms. In contrast, serum CK is elevated in only 63% of female patients.

Mental retardation is usually mild and is present in 70% of male patients. In the study by Sugie *et al*. [13•], there was only one female patient with mental retardation. Brain magnetic resonance imaging is usually normal. In two autopsy cases, we found vacuolar changes in the

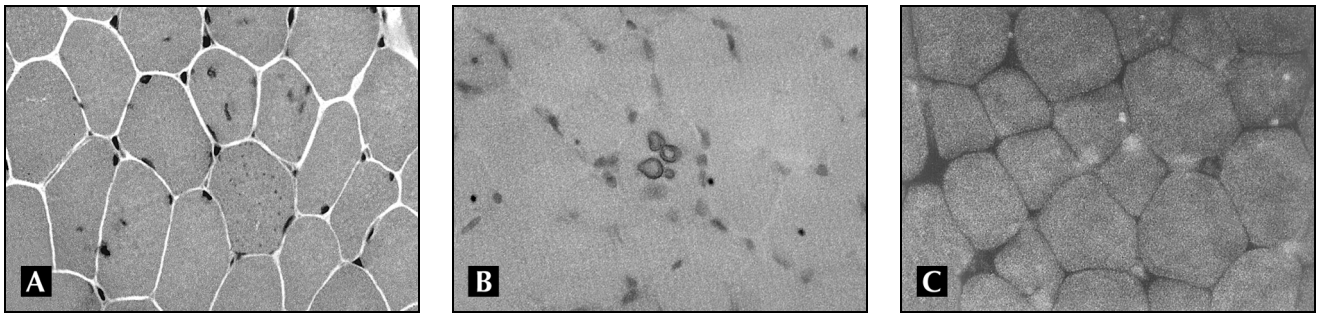

Figure 3. Muscle pathology of Danon disease. **A,** Autophagic vacuoles are so tiny that they look more like solid granules on hematoxylin and eosin staining. **B,** Vacuolar membranes have acetylcholine esterase activity, and thus sarcolemmal features. **C,** Lysosome-associated membrane protein-2 (LAMP-2) is completely absent when immunostaining is performed.

cytoplasm of red nucleus; however, this abnormality does not directly account for the mental retardation.

Muscle biopsies show many scattered intracytoplasmic vacuoles, which on hematoxylin and eosin staining often look like tiny basophilic granules, in addition to mild-to-moderate fiber size variation (Fig. 3A). Usually, no necrotic or regenerating fibers are seen. Interestingly, the vacuolar membranes show activity for acetylcholinesterase and nonspecific esterase (Fig. 3B) [14]. Acetylcholinesterase is present in specialized sarcolemma at the neuromuscular junction called junctional folds. Therefore, the presence of acetylcholinesterase activity indicates that the vacuolar membrane has features of sarcolemma. This characteristic has been confirmed by immunohistochemical study for other sarcolemma-specific proteins, including dystrophin, sarcoglycans, dystroglycans, and laminin [14,15].

By electron microscopy, the intracytoplasmic vacuoles typically contain myelin figures, electron-dense bodies, and various cytoplasmic debris; therefore, they are autophagic vacuoles. Interestingly, basal lamina is sometimes seen along the inner surface of autophagic vacuoles, providing further evidence that the vacuolar membrane has features of sarcolemma. Occasionally, sarcolemma and vacuolar membranes appear to be connected, giving an appearance similar to fiber splitting.

By immunohistochemical and Western blot analyses, LAMP-2 protein is absent in the skeletal muscles regardless of the specific *LAMP-2* gene mutation (Fig. 3C). Western blot analysis of the cardiac muscle in one patient also showed a complete absence of LAMP-2 protein. In contrast, other lysosomal membrane proteins, such as lysosomal integral membrane protein-I, are associated with the autophagic vacuoles in Danon disease.

Lysosome-associated membrane protein-2 is a type 1 membrane protein with a large, luminal domain connected to a transmembrane region and a short cytoplasmic tail. The luminal domain is heavily glycosylated; most of the potential N-linked glycosylation sites are utilized, yielding a molecular mass of 90 to 120 kD for the approximately 40-kD core protein. LAMP-2 is abundantly expressed and is thought to coat the inner surface of the lysosomal membrane together with its autosomal paralogue, LAMP-1. Therefore, LAMPs are thought to protect lysosomal membrane, and thus cytoplasm, from proteolytic enzymes within the lysosomes.

The cytoplasmic tail of LAMP-2 is short, consisting of only 11 amino acids, but has a well-conserved tyrosine residue that is thought to provide a crucial signal for trafficking of LAMP-2 molecules to lysosomes. Moreover, the cytoplasmic tail of LAMP-2 is thought to function as a receptor for the uptake of certain proteins into lysosome for degradation in association with the 73-kD heat shock cognate protein.

The expression of LAMP-2 is increased in a variety of situations, whereas LAMP-1 seems to be expressed constitutively; therefore, expression of LAMP-2 is likely to be specifically regulated [16]. Interestingly, a small fraction (2% to 3%) of LAMP-2 is present in the plasma membrane and its expression in the cell surface is increased in certain situations, including malignancy and scleroderma. Furthermore, LAMP-1 has recently been shown to have a role in fusion of the lysosomal membrane and plasma membrane [17]. Most likely, LAMP-2 also has a role in the fusion of the membranes, and this may be related to the development of the unusual autophagic vacuoles with sarcolemma features.

The *LAMP-2* gene is located on Xq24, whereas the gene *LAMP-1* is on 13q34. The *lamp-2* open reading frame consists of 1233 nucleotides and encodes 410 amino acids. Exons 1 through 8 and part of exon 9 encode a luminal domain, whereas the remainder of exon 9 encodes both a transmembrane domain and a cytoplasmic domain. Human exon 9 exists in two forms, 9a and 9b, that are alternatively spliced and produce two isoforms, LAMP-2a and LAMP-2b, respectively. LAMP-2a is expressed rather ubiquitously whereas LAMP-2b is expressed specifically in heart and skeletal muscles.

Genetically confirmed Danon disease patients have been ethnically diverse, suggesting that this disorder can be seen in any ethnic group. Most of the mutations identified so far are stop-codon or out-of-frame mutations that are predicted to truncate the protein, resulting in loss of the transmembrane and cytoplasmic domains. Therefore, the mutated products cannot function as a lysosomal membrane protein.

Danon disease is clinically uniform in male patients. No apparent genotype-phenotype correlation has been observed except for a patient who had exon 9b mutation, which is predicted to affect only LAMP-2b isoform, one of two LAMP-2 isoforms. The mutation in this patient not only suggests that LAMP-2b is the major isoform in cardiac and skeletal muscles, but also suggests that a deficiency of LAMP-2b by itself is sufficient to cause the disease, albeit in a milder form.

X-linked myopathy with excessive autophagy

In 1988, Kalimo *et al*. [18] reported a new type of autophagic vacuolar myopathy in a Finnish family. The disease is transmitted in an X-linked recessive manner. Clinically, the disease is characterized by slowly progressive muscle weakness and atrophy that spares cardiac and respiratory muscles. Muscle biopsy shows many tiny vacuoles, and the vacuolar membranes have features of plasma membrane as in Danon disease. Autophagic vacuoles are seen in the cytoplasm. The muscle pathology resembles that of Danon disease; therefore, the two diseases are likely to share similar molecular pathomechanisms.

The characteristic pathologic findings in X-linked myopathy with excessive autophagy (XMEA) are depositions of complement C5b-9 over the surface of muscle fibers and multilayered basal lamina along the sarcolemma, which are not seen in Danon disease. Furthermore, the presence of LAMP-2 in XMEA muscle clearly demonstrates that XMEA is distinct from Danon disease. In addition, the XMEA locus has been mapped to Xq28, whereas the gene encoding LAMP-2 is present on Xq24.

Infantile autophagic vacuolar myopathy

There have been two well-documented reports of infants with autophagic vacuolar myopathy described as having the infantile form of "lysosomal glycogen storage disease with normal acid maltase" [19]. Both patients presented with muscle weakness and hypotonia at birth and died early in their lives. Muscle biopsies showed extensive vacuolar changes with increased glycogen, but acid maltase activity was normal in both patients; hence, the diagnosis seemed to be clear. Nevertheless, the infantile disease is distinct from Danon disease because LAMP-2 protein is not deficient in the skeletal muscle and sequences of the *LAMP-2* gene are normal. Interestingly, as in XMEA muscle, complement C5b-9 stained muscle sarcolemma in one infant. On electron microscopy, many vacuoles containing membrane-bounded glycogen particles, free glycogen particles, and cytoplasmic degradation products scattered in the cytoplasm. In addition, duplication of basal lamina into two layers was observed along portions of the sarcolemma. Multilayered basal lamina was also seen in some fibers. Material exocytosed from vacuoles accumulated under and between the multiple layers of basal lamina. The deposition of complement C5b-9 over the surface of muscle fibers and the multiplication of basal lamina suggest that the pathologic features of infantile autophagic vacuolar myopathy are more similar to those of XMEA than Danon disease.

Conclusions

Unlike muscle diseases characterized by the presence of rimmed vacuoles, in autophagic vacuolar myopathies the primary defects are likely to reside in lysosomes, as has been demonstrated in Danon disease and acid maltase deficiency. XMEA and infantile autophagic vacuolar myopathy show unusual autophagic vacuoles with sarcolemmal features and, therefore, are likely to belong to a distinct group of myopathies.

Although the concept of autophagic vacuolar myopathy or lysosomal myopathy is not yet well established, most likely there will be other diseases discovered in this group.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Alberts B: *Molecular Biology of the Cell,* edn 4. New York: Garland Science; 2001.
2. Nonaka I, Sunohara N, Ishiura S, *et al.*: **Familial distal myopathy with rimmed vacuole and lamellar (myeloid) body formation.** *J Neurol Sci* 1981, **51:**141–155.
3. Argov Z, Yarom R: **'Rimmed vacuole myopathy' sparing the quadriceps: a unique disorder in Iranian Jews.** *J Neurol Sci* 1984, **64:**33–43.
4. Ikeuchi T, Asaka T, Saito M, *et al.*: **Gene locus for autosomal recessive distal myopathy with rimmed vacuoles maps to chromosome 9.** *Ann Neurol* 1997, **41:**432–437.
5. Mitrani-Rosenbaum S, Argov Z, Blumenfeld A, *et al.*: **Hereditary inclusion body myopathy maps to chromosome 9p1-q1.** *Hum Mol Genet* 1996, **5:**159–163.
6. •• Eisenberg I, Avidan N, Potikha T, *et al.*: **The UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene is mutated in recessive hereditary inclusion body myopathy.** *Nat Genet* 2001, **29:**83–87.

This paper reports that *GNE* gene is implicated in hereditary inclusion body myopathy.

7. Nishino I, Noguchi S, Murayama K, *et al.*: **Distal myopathy with rimmed vacuoles is allelic to hereditary inclusion body myopathy.** *Neurology* 2002, in press.
8. McFerrin J, Engel WK, Askanas V: **Impaired innervation of cultured human muscle overexpressing beta APP experimentally and genetically: relevance to inclusion-body myopathies.** *Neuroreport* 1998, **9:**3201–3205.
9. • Sugarman MC, Yamasaki TR, Oddo S, *et al.*: **Inclusion body myositis-like phenotype induced by transgenic overexpression of bAPP in skeletal muscle.** *Proc Natl Acad Sci U S A* 2002, **99:**6334–6339.

This paper suggests that amyloid deposition may be responsible for inclusion body mysositis pathology, although abnormalities of lysosomes are not described.

10. DiMauro S, Musumeci O: **Metabolic myopathies.** In *Neuromuscular Disorders in Clinical Practice.* Edited by Katirji B. Boston: Butterworth-Heinemann; 2002.
11. Danon MJ, Oh SJ, DiMauro S, *et al.*: **Lysosomal glycogen storage disease with normal acid maltase.** *Neurology* 1981, **31:**51–57.

12.•• Nishino I, Fu J, Tanji K, *et al.*: **Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease).** *Nature* 2000, **406:**906–910.

The autophagic vacuolar myopathy with unique vacuolar membrane was shown to be due to the deficiency of lysosome-associated membrane protein-2 (LAMP-2). This finding was also confirmed by LAMP-2 knockout mouse in another report in the same issue of the jounal.

13.• Sugie K, Yamamoto A, Murayama K, *et al.*: **Clinicopathological features of genetically confirmed Danon disease.** *Neurology* 2002, **58:**1773–1778.

Clinicopathologic review of Danon disease.

14. Murakami N, Goto YI, Itoh M, *et al.*: **Sarcolemmal indentation in cardiomyopathy with mental retardation and vacuolar myopathy.** *Neuromusc Disord* 1995, **5:**149–155.

15. Muntoni F, Catani G, Mateddu A, *et al.*: **Familial cardiomyopathy, mental retardation and myopathy associated with desmin-type intermediate filaments.** *Neuromusc Disord* 1994, **4:**233–241.

16. Kannan K, Divers SG, Lurie AA, *et al.*: **Cell surface expression of lysosome-associated membrane protein-2 (lamp2) and CD63 as markers of in vivo platelet activation in malignancy.** *Eur J Haematol* 1995, **55:**145–151.

17. Reddy A, Caler EV, Andrews NW: **Plasma membrane repair is mediated by Ca2+-regulated exocytosis of lysosomes.** *Cell* 2001, **106:**157–169.

18. Kalimo H, Savontaus ML, Lang H, *et al.*: **X-linked myopathy with excessive autophagy: a new hereditary muscle disease.** *Ann Neurol* 1988, **23:**258–265.

19. Yamamoto A, Morisawa Y, Verloes A, *et al.*: **Infantile autophagic vacuolar myopathy is distinct from Danon disease.** *Neurology* 2001, **57:**903–905.