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Late-adult onset of X-linked myopathy with excessive autophagy (XMEA)

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Abstract

Introduction—X-linked myopathy with excessive autophagy (XMEA) is characterized by autophagic vacuoles with sarcolemmal features. Mutations in *VMA21* result in insufficient lysosome acidification, causing progressive proximal weakness with onset before age 20 and loss of ambulation by middle age.

Methods—We describe a patient with onset of slowly progressive proximal weakness of the lower limbs after age 50 who maintains ambulation with the assistance of a cane at age 71.

Results—Muscle biopsy at age 66 showed complex muscle fiber splitting, internalized capillaries, and vacuolar changes characteristic of autophagic vacuolar myopathy. Vacuoles stained positive for sarcolemmal proteins, LAMP2, and complement C5b-9. Ultrastructural evaluation further revealed basal lamina reduplication and extensive autophagosome extrusion. Sanger sequencing identified a known pathologic splice site mutation in *VMA21* (c.164–7T>G).

Conclusions—This case expands the clinical phenotype of XMEA and suggests *VMA21* sequencing be considered in evaluating men with LAMP2-positive autophagic vacuolar myopathy.

Keywords

Autophagic vacuolar myopathy; autophagic vacuoles with sarcolemmal features; X-linked myopathy with excessive autophagy; *VMA21*; progressive proximal muscle weakness

INTRODUCTION

The process of autophagocytosis is a highly regulated means of providing cells with sources of energy via protein and organelle degradation by lysosomes.¹ While proper functioning of the pathway plays a critical role in processes ranging from development to basal cellular homeostasis, aberrant functioning has been implicated in a number of pathological states.² It has been shown that autophagy is prominent in skeletal muscle, and abnormalities of autophagic activity have been demonstrated to cause neuromuscular disorders known collectively as autophagic vacuolar myopathies (AVM).^{3,4} A sub-group of AVM has been distinguished by the presence of autophagic vacuoles with sarcolemmal features.^{5,6} These vacuoles are rimmed by membranes containing sarcolemmal proteins and can be found in myopathies that include Danon disease and X-linked myopathy with excessive autophagy (XMEA).⁷

Lysosomal-associated membrane protein 2 (LAMP2) plays a critical role in the autophagocytic pathway via facilitation of autophagosome-lysosome fusion.^{2,8,9} Danon disease, caused by mutations in the *LAMP2* gene, is an X-linked dominant condition that results in severely affected young men and mid-life onset of cardiac symptoms in carrier women.^{6,7} The triad of cardiomyopathy (hypertrophic or dilated), myopathy, and mental retardation is the classic presentation of Danon disease before age 20.¹⁰ Cardiomyopathy in males is often severe, resulting in rapid deterioration and death in patients less than age 30 years; heart transplantation is the most effective therapeutic intervention.¹⁰⁻¹² While mild, the myopathy associated with Danon disease can be observed in 90% of males and affects primarily neck and shoulder girdle muscles.⁷ Serum CK is elevated (1574 $\pm$ 790 U/L) regardless of apparent muscle symptomology.¹⁰

Originally identified in 1988, XMEA is a rare X-linked myopathy with typical onset before age 20.¹³⁻¹⁵ Proximal muscles display a slow, progressive weakness and atrophy, and those affected typically lose independent ambulation after age 50.¹⁵ Mutations in the gene coding for vacuolar membrane ATPase activity 21 (VMA21) were determined to be the cause of XMEA.¹⁵ VMA21 is an assembly chaperone for the principal mammalian proton pump required for lysosome acidification.¹⁵ Loss of appropriate VMA21 activity leads to the formation of autophagic vacuoles with sarcolemmal features and shares many histopathologic characteristics with Danon disease, but with the additional observation of multilayered basal laminae and deposition of complement C5b-9 along the vacuolar and cell surface membranes.^{6,15}

We describe an XMEA patient with progressive proximal weakness of the lower limbs that started near age 55. Despite the late onset of symptoms, a muscle biopsy displayed the classic histopathology of autophagic vacuoles with sarcolemmal features and complement C5b-9 deposition, and Sanger sequencing revealed a known pathologic splice site mutation in the *VMA21* gene. The late clinical presentation and maintenance of ambulation past age 70 expand the phenotype of XMEA.

MATERIALS & METHODS

Patient

The patient presented at age 65 with chief complaint of slowly progressive proximal lower limb weakness beginning near age 55. There were no associated symptoms of myalgia, cramps, contractures, cardiac dysfunction, cognitive disturbance, or sensory abnormalities. He participated in high school sports and remained physically active through mid-adulthood. Repeated serum CK values were only elevated mildly (300 – 400 u/l). His parents lived into their late 70s without evidence of weakness. An older brother had onset of muscle weakness in his mid-40s and was diagnosed with myositis. The myositis was not responsive to treatment, and he began using a wheelchair in his late 50s. He died at age 61 due to trauma sustained in an automobile accident. His muscle biopsy slides could not be located. No other immediate or remote family members are affected. On exam, our patient had proximal weakness of the lower limbs more than the upper limbs. Medical Research Council scale strength was deltoids 4+, biceps 5–, triceps 4+, infraspinatus 5–, pectoralis major 4+, hip flexion 3+, hip extension 3, thigh adduction 4+, thigh abduction 4+, knee extension 3, and knee flexion 5. There was no scapular winging. Except for the hamstrings, muscle bulk was decreased in proximal upper and lower extremity muscles. Thoracic paraspinal muscle bulk was normal. Distal muscle strength and bulk was normal. No contractures were noted. Facial and neck muscle strength was normal. Concentric needle EMG demonstrated increased insertional activity, positive sharp waves, and fibrillation potentials in affected muscles examined. Complex repetitive discharges were not seen. Voluntary motor unit action potentials were decreased amplitude, normal to decreased duration, and polyphasic with an early recruitment pattern. The patient is now 71 years old and remains ambulatory with the assistance of leg braces and a single-prong cane. His disease progression continues to be slow.

Histology

A biceps brachii muscle biopsy was obtained at age 66. Cryosections were stained with H&E, NADH, Gomori trichrome, and ATPase and were examined by routine light microscopy. Enzyme histochemistry was also performed for acetylcholinesterase (AChE). Immunofluorescence assessment was performed using antibodies against dystrophin [Abcam and Developmental Studies Hybridoma Bank (DSHB), The University of Iowa], dystroglycans (DSHB), sarcoglycans (DSHB and Leica), nNOS (Leica), spectrin (Leica), merosin (Leica), collagen VI (DSHB), perlecan (Millipore), dysferlin (Leica), MHC class I (DAKO), LAMP1 and LAMP2 (DSHB), and complement C5b-9 (Abcam). Electron microscopy was performed using standard techniques.

Mutation screening of VMA21

DNA extraction from blood was performed using DNAeasy Blood & Tissue Kit from Qiagen. Three separate PCR reactions were used to amplify the 3 exons. The primers were previously used to identify *VMA21* mutations in XMEA patients.¹⁵ The PCR reactions were carried out using a standard protocol. The PCR products were visualized on a 1.5% agarose gel and purified using microCLEAN (Microzone). Quantification was performed using NanoDrop ND-1000 spectrophotometer V 3.3 (Thermoscientific). PCR products (50 ng

each) were used for Sanger sequencing, with the forward and reverse primers at a concentration of 5 pmol each in a final volume of 7.7 μ L. The patient's mutation was confirmed by sequences originating from upstream and downstream primers.

RESULTS

Muscle biopsy analysis

Standard histochemistry and enzyme histochemistry revealed numerous cytoplasmic vacuoles in a large number of muscle fibers. Central and peripheral vacuolar pathology was evident with H&E. Internalized capillaries and complex fiber splitting were also observed throughout the sample. Endomysial fibrosis and fatty infiltration were widespread, and angulated atrophic fibers and pyknotic nuclear clusters were also readily apparent (Fig. 1A–D). Inflammatory cell infiltrates were absent, but scattered fibers were undergoing necrosis or regeneration. The fiber type distribution appeared normal. Enzyme histochemical staining for AChE accentuated the numerous vacuole-containing fibers (Fig. 1E). Vacuolar inclusions displaying abundant AChE positivity in their membranes were observed at the periphery of the muscle fibers and throughout the sarcoplasm (Fig. 1F–H). No inclusion bodies or ragged-red fibers were identified.

Immunofluorescence revealed positive staining for dystrophin at the periphery of the muscle fibers, along muscle fiber splits, and rimming the vacuoles (Fig. 2A). Vacuoles were found to stain positively for the extracellular matrix protein perlecan but were negative for collagen VI (Fig. 2B). The sarcolemmal and T-tubule protein dysferlin was strongly positive in the vacuolar and muscle fiber surface membranes (Fig. 2C). Vacuoles were also rimmed by strongly positive staining for caveolin-3, alpha- and beta-dystroglycan, sarcoglycans, nNOS, and spectrin (data not shown), while only weakly positive staining for merosin (laminin alpha2) was observed (data not shown). Vacuoles stained intensely for the lysosomal membrane proteins LAMP2 and LAMP1 (Fig. 2D). Assessment of MHC class I showed expression throughout the muscle biopsy with regions of stronger positivity (Fig. 2E). Higher magnification of these latter areas revealed that MHC I expression was localized to the membranes of both the muscle fiber sarcolemma and the internal vacuoles (Fig. 2F). Complement C5b-9 (membrane attack complex) was widely distributed through the muscle tissue, localizing to the vacuoles as well as the muscle fiber surface membranes (Fig. 2G–H).

Electron microscopy revealed large, debris-containing intracellular vacuolar inclusions that focally disrupted the sarcomeric structure of the muscle fibers (Fig. 3A). These vacuoles were sometimes associated with redundant membranous material (Fig. 3A–B). Ultrastructural analysis further revealed that these autophagic vacuoles were extruded from the cell at various points throughout the sample and were associated with a serrated cell surface with or without duplicated basement membranes (Fig. 3C–D).

Mutation analysis

Sanger sequencing of exons and exon-intron boundaries of *VMA21* revealed a previously reported detrimental substitution of a pyrimidine into a purine, c.164-7T>G, in the strictly conserved polypyrimidine tract, upstream of the third exon.

DISCUSSION

X-linked myopathy with excessive autophagy (XMEA) is characterized pathologically by the presence of vacuoles in skeletal muscle that stain positively for a number of sarcolemmal proteins.^{6,7} The vacuolar changes evident in the muscle cells of patients with XMEA are similar to those observed in Danon disease, but XMEA has the additional features of membrane-associated complement C5b-9, calcium deposition, and basal lamina reduplication.^{16,17} Routine analysis of our patient's muscle biopsy suggested an autophagic vacuolar myopathy. Enzyme histochemistry and immunofluorescence staining revealed autophagic vacuoles with sarcolemmal features and extensive sarcolemmal C5b-9 deposition. Positive immunostaining for LAMP2 excluded the diagnosis of Danon disease. Ultrastructurally, numerous lysosomes and autophagosomes as well as redundant membranes and extrusion of autophagic debris were noted. Thus, the muscle biopsy was interpreted to show a late-onset variant of XMEA.

XMEA is an X-linked autosomal recessive disorder mapped to the *VMA21* gene on Xq28 that is caused by hypomorphic mutations leading to formation of a dysfunctional VMA21 protein product.^{15,18-21} Sanger sequencing of *VMA21* in our patient revealed a mutation (c.164-7T>G) reported previously in 3 French families and more recently in a Japanese family; in each of these XMEA cases, onset of weakness occurred much earlier than the age of onset observed in our patient.^{15,22} This mutation was demonstrated to interfere with a polypyrimidine tract of a splice site via introduction of a purine base, resulting in a decrease in U2AF splice factor binding.¹⁵ The physiological effect of this hypomorphic mutation is decreased activity of VMA21 and reduced lysosomal acidification, which presumably leads to accumulation of vacuoles through both increased formation and delayed progression through the degradative pathway.¹⁵ The presence of this previously identified mutation indicates that our patient is manifesting a late-onset form of XMEA rather than presenting with a novel disease state.

As currently described in the literature, XMEA presents as progressive muscle weakness in the proximal lower limbs of men. Symptoms generally begin within the first 2 decades of life with slow progression to include the upper limbs and distal muscles without the cardiomyopathy seen in Danon disease.^{13,14} Despite the similarities in pathological findings and underlying genetic cause, the age of onset in our patient was considerably later than previously identified patients with XMEA.¹⁵ While there have been reports of late-onset autophagic vacuolar myopathy with sarcolemmal features, these patients presented with involvement of additional organ systems not affected in our patient.^{23,24} The late onset of classic XMEA symptoms in our patient compared to the children described previously with the identical mutation suggests there may be modifying factors beyond the *VMA21* mutation, perhaps genetic or environmental, influencing disease onset.

Here we describe a novel case of late adult-onset XMEA with a previously identified disease-causing mutation in *VMA21* that manifests the muscle biopsy findings very similar to childhood-onset XMEA. While this disease generally results in onset of weakness by the second decade and loss of independent ambulation after age 50, the patient described here retained a high level of physical activity through mid-adulthood and did not develop weakness until his mid-50s. Now age 71 years, the patient remains ambulatory. This report expands the clinical phenotype associated with XMEA, suggesting that *VMA21* sequencing should be considered in the diagnostic workup of men who present with LAMP2-positive autophagic vacuolar myopathy.

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ABBREVIATIONS

AChE	acetylcholinesterase
AVM	autophagic vacuolar myopathy
CK	creatine kinase
CRD	complex repetitive discharges
EMG	electromyography
H&E	hematoxylin and eosin
LAMP2	lysosomal-associated membrane protein 2
MHC	major histocompatibility complex
NADH	nicotinamide adenine dinucleotide
NCS	nerve conduction studies
nNOS	neuronal nitric oxide synthase
PCR	polymerase chain reaction
U2AF	U2 auxillary factor
VMA21	vacuolar membrane ATPase activity 21
XMEA	X-linked myopathy with excessive autophagy.

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Figure 1. Muscle biopsy histopathology

Cryosections stained with H&E (panels A–D) or by acetylcholinesterase (AChE) enzyme histochemistry (panels E–H) show numerous cytoplasmic vacuoles, complex muscle fiber splitting, and internalized capillaries. Neuropathic features of angulated atrophic fibers in small groups (B, C) and pyknotic nuclear clusters (B, white arrowhead) are also evident. One of the internalized capillaries is indicated by the black arrowhead in panel C.

Hypertrophic muscle fibers in panels D and F (black arrows) have numerous peripherally located vacuoles, possibly extrusion of autophagosomes. Scale bar = 50 μm in panels A–D and F–H; scale bar = 500 μm in panel E.

Figure 2. Immunofluorescence studies

Various sarcolemma-associated proteins are shown to rim cytoplasmic vacuoles. Dystrophin is illustrated in panel A. The basement membrane protein perlecan (red) is present at the periphery of muscle fibers, rims cytoplasmic vacuoles, and co-localizes with collagen VI (green) along splits, around internalized capillaries (B). Dysferlin localizes with cytoplasmic vacuoles in addition to its normal position at the sarcolemma and ttubule system (C). LAMP2 is strongly positive, especially in association with cytoplasmic vacuoles (D). MHC class I expression is widespread and involves both the sarcolemma and cytoplasmic vacuoles (E, F). Complement C5b-9 deposition has a similar distribution (G, H). Scale bar = 50 μm in panels A–D, F and H; scale bar = 200 μm in panel E; scale bar = 500 μm in panel G.

Figure 3. Ultrastructural pathology

Electron microscopy shows abundant lysosomes/autophagosomes within the cytoplasm (A, B) and along the periphery (C, D, double arrows) of many muscle fibers. Redundant membranes are a prominent feature of some cytoplasmic vacuoles (B). Autophagosome extrusion (black arrowheads in D) has created a serrated surface (C) or led to reduplication of sarcolemmal basement membranes (D, arrows) in some muscle fibers. Scale bar = 1.5 μm in panel A; scale bar = 0.75 μm in panel B; scale bar = 1 μm in panels C and D.