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Case report

Cardiac autophagic vacuolation in severe X-linked myopathy with excessive autophagy

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Abstract

X-linked myopathy with excessive autophagy (XMEA), caused by mutations of the *VMA21* gene, is a strictly skeletal muscle disease. Extensive studies in yeast established VMA21 as the master assembly chaperone of V-ATPase, the complex multisubunit proton pump that acidifies organelles and that is vital to all mammalian tissues. As such, skeletal muscle disease exclusivity in XMEA is highly surprising. We now show that the severest *VMA21* mutation, c.164-6t>g, does result in XMEA-typical pathology with autophagic vacuolar changes outside skeletal muscle, namely in the heart. However, even patients with this mutation do not exhibit clinical extramuscular disease, including cardiac disease, despite extreme skeletal muscle wasting to the extent of ventilation dependence. Uncovering the unique skeletal muscle vulnerability to defective organellar acidification, and resultant tissue-destructive excessive autophagy, will be informative to the understanding of muscle physiology. Alternatively, understanding extramuscular resistance to *VMA21* mutation might disclose heretofore unknown mammalian V-ATPase assembly chaperones other than VMA21.

Keywords: XMEA; LAMP2; Autophagy; Lysosome; Congenital; CAVM; Acidification

1. Introduction

We recently published a paper in *Neuromuscular Disorders* titled: 'No cardiomyopathy in X-linked myopathy with excessive autophagy' [1]. This was a comprehensive electrocardiography, echocardiography and gadolinium-enhanced cardiac MRI study in five members (ages 25–48 years) and cardiac autopsy in one member of the original XMEA family [2]. No cardiac abnormalities were present, including no autophagic vacuolation under light or electron microscopy, which in skeletal muscle is the defining feature of the disease. Absence of even subclinical changes in the heart accentuated the puzzling aspect of XMEA of apparent non-involvement of extra-skeletal muscle organs. The disease is caused by mutations of a gene (*VMA21*) that encodes the chief assembly chaperone of the V-ATPase proton pump which is vital to all cells, yet to date pathology has been reported only in skeletal myofibers, leukocytes and fibroblasts, and clinical disease only in skeletal muscle [3].

All XMEA mutations are hypomorphic alleles that reduce but do not eliminate *VMA21* expression, most of them intronic variants that act by reducing splicing efficiency [3]. Most XMEA patients have a uniform clinical course as described in the original family, namely insidious onset of limb girdle weakness sometime in childhood with slow progression toward wasting and ambulation loss after the fifth decade [2–5]. Few patients, described more recently, have a much more severe course with infantile or neonatal onset, sometimes with death in infancy due to respiratory failure, minimal or no ambulation, and artificial ventilation dependence. These patients have more severe mutations, resulting in greater reductions in *VMA21* expression and V-ATPase activities, than those with classical XMEA [6,7]. Members of the worst affected family (Congenital Autophagic Vacuolar Myopathy (CAVM); mutation c.164-6t>g) have a mild cardiac hypertrophy on echocardiography, but additional testing has not been performed, and whether their hearts exhibit vacuolation is unknown [7].

2. Case report

In 1998 the case of a hypotonic male infant was reported, with ventilation dependence, severe muscle weakness, and

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death by 27 months from respiratory insufficiency [8]. His skeletal muscle pathology (electron-dense-debris-filled vacuoles, deposition of complement membrane attack complex on vacuoles and multiplication of the basal lamina) resembled more closely the pathology of XMEA than that of Danon disease, the vacuolar myopathy that most closely resembles XMEA. Following identification of *LAMP2* as the Danon disease gene, he was shown to have no *LAMP2* mutations and normal LAMP2 protein in muscle, which exclude Danon disease [8,9]. Upon his death permission was obtained for a cardiac autopsy, and the heart showed slight hypertrophy and 'scattered fibers with autophagic vacuoles on electron microscopy' [8]. Examination of the electron micrograph provided with the original publication shows this cardiac vacuolation to exactly resemble that of XMEA skeletal muscle. To determine whether this old case was in fact an XMEA case, we sequenced his DNA and found the same *VMA21* c.164-6t>g mutation as in our severest XMEA family mentioned above.

3. Discussion

The V-ATPase is a complex proton pump composed of over 13 subunits. It is ubiquitous to all cells, using energy from ATP to pump protons and acidify organelles, including lysosomes, autolysosomes, the Golgi apparatus and others. It is evolutionarily related to the P-ATPase, the last complex of the mitochondrial electron transport chain, through which protons flow in the reverse direction to generate, rather than consume, ATP. Detailed work in yeast identified VMA21 as the master assembly chaperone of the V-ATPase [10]. When *VMA21* was established as the XMEA gene, the question arose as to why only skeletal muscle is affected. Even the heart was unaffected, even with electron microscopic analysis [1]. When patients with the severest form of XMEA were described (mutation c.164-6t>g associated with neonatal lethality or profound generalized skeletal muscle wasting in surviving newborns), they too did not have overt cardiac disease – cardiac tissue was unavailable from these patients for microscopic analysis [7]. The present study shows that this severe mutation (c.164-6t>g) does in fact manifest the typical electron microscopic XMEA changes in the heart, albeit subclinically. VMA21 therefore plays at least some role in the heart, but the question remains as to why the heart, and other extramuscular organs, are at most so minimally affected with mutation of the assembly chaperone of the proton pump vital to all tissues.

The present pathogenetic model of XMEA is as follows: Reduced V-ATPase raises lysosomal pH, which impairs the final degradative stage of autophagy. This failure upregulates autophagy, at least in part through mTOR signaling, and drives proliferation of autolysosomes, which in turn are unable to complete autophagy and merge together to form the large debris-filled vacuoles that characterize the disease [3]. *VMA21* mutations reduce V-ATPase activity by 75% in classical XMEA and by 90% in the severest form of the disease (CAVM) (c.164-6t>g), as determined by measurements in biopsied skeletal muscle and lymphoblasts [3,7]. Possibly, extramuscular organs tolerate this degree of reduction, but would succumb to deeper diminution of V-ATPase activity. In fact there are no known patients with complete loss-of-function mutations. Another possibility is that XMEA mutations, most of which affect splicing, do so to a lesser extent in extramuscular tissues allowing higher levels of *VMA21* expression. However, this possibility is less favored, because extramuscular cells tested, lymphoblasts, have even lower residual V-ATPase activity than skeletal muscle from the same patients [3,7]. Finally, it is possible that in mammalian tissues there are assembly chaperones other than the one, VMA21, identified through studies of yeast.

As mentioned, Danon disease is the closest myopathological differential diagnosis to XMEA. One of the major clinical differences between the two diseases is the early and severe involvement of the heart in the former, generally requiring transplantation. Multiple functions have been attributed to the LAMP2 protein, some of which indirectly overlap the known function of VMA21. LAMP2 is involved in intracellular motility of lysosomes, maintenance of the lysosome acidic environment, fusion of lysosomes with autophagosomes, and maturation of autolysosomes [11]. It is likely that the shared autophagic vacuolation between the two diseases stems at least in part from their shared effects on lysosomal and autolysosomal acidification. But why is the heart so majorly affected in Danon disease and not XMEA? Both are X-linked diseases affecting primarily males. Most Danon disease mutations lead to complete loss of the protein's function, while all XMEA mutations, even the severest (CAVM), leave residual activity. Possibly, complete loss of VMA21, if compatible with life, would result in just as severe cardiac pathology as occurs in Danon disease. On the other hand, females with Danon disease, who usually have 50% preserved LAMP2 function, usually have only cardiac disease (and no neurological or skeletal muscle disease) [11], indicating that it is not the complete loss of LAMP2 function alone that underlies the cardiac disease in males. Likely, functions of LAMP2 other than lysosomal acidification, such as chaperone-mediated autophagy and endosomal cholesterol transport [11], contribute to the cardiomyopathy.

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