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CONGENITAL AUTOPHAGIC VACUOLAR MYOPATHY IS ALLELIC TO X-LINKED MYOPATHY WITH EXCESSIVE AUTOPHAGY

X-linked myopathy with excessive autophagy (XMEA) is characterized by weakness and wasting primarily of the proximal muscles of the lower extremities. Onset is usually after age 5 and progression is extremely slow with ambulation maintained well into the 50s. The heart, CNS, peripheral nervous system, and other organs are clinically spared. Pathology reveals large autophagic vacuoles enclosing incompletely degraded cytoplasmic components, which translocate to the myofiber surface and extrude their contents, forming a field of cell debris between multiplied layers of basal lamina.¹ XMEA is caused by mutations of the *VMA21* gene, which reduce, but do not eliminate, expression of the chief assembly chaperone (VMA21) of the main proton pump (V-ATPase [vacuolar-type H⁺-adenosine triphosphatase]) of all mammalian cells.²

A neurologic cause is present in 2 per 10,000 term neonates with ventilation-requiring respiratory failure,³ including multiple genetic neuromuscular diseases. Among these is a congenital autophagic vacuolar myopathy (CAVM) with myopathologic features resembling XMEA.⁴ We here revisit the original CAVM family 10 years after its description and show that it is allelic to XMEA. Clinical details are in the original publication.⁴ Briefly, 7 male members were affected. Of the seven, 5 died soon after birth due to inability to breathe and suckle, one was saved by intubation-ventilation and nasogastric feeding, and the other by nasogastric feeding alone. Both were hypotonic with 10-fold-elevated creatine kinase. They walked late (approximately age 2 years) and only until age 5, becoming wheelchair-bound due to weakness. Presently aged 18 and 20 years, they are wheelchair-bound with profound generalized muscle wasting and severe scoliosis (figure, A), and both are on nocturnal noninvasive ventilation. Although dysphagic and dysarthric, they still feed orally. Peripheral nervous system and CNS are unaffected, and both attend college. Mild cardiac hypertrophy is present, without significant ECG abnormalities. Skeletal muscle pathology reveals autophagic vacuolation (figure, B, and original report⁴).

All methods are as detailed previously.² We sequenced *VMA21* exons and exon-intron boundaries in the 2 brothers. In the strictly conserved polypyrimidine tract upstream the third exon, we found a pyrimidine to purine substitution, c.164–6t>g (figure, C and D). None of 949 control chromosomes (including 192 ethnically matched) had this variant, and the variant is not present in any public or in-house sequence databases. It is predicted to reduce splicing efficiency by interfering with binding of the U2 auxiliary splice factor, which requires a continuous stretch of pyrimidines upstream spliced exons.^{2,5} Reduced splicing efficiency would result in abnormal messenger RNA (mRNA) spliceforms and/or reduced mRNA amounts. Reverse transcription (RT)-PCR in lymphoblasts revealed no abnormal transcripts. Semi-quantitative and quantitative RT-PCR showed reduced *VMA21* expression (figure, E and F). Considering the question of why the patients with CAVM are so much more severely affected than patients with XMEA, we included patients with XMEA in the quantitative RT-PCR experiment. While in XMEA, *VMA21* mRNA was reduced to 45% to 69% of normal, in CAVM, it was decreased to 22% to 25% of normal (figure, F). Finally, we measured V-ATPase activity. In XMEA, V-ATPase activity is reduced to 16% to 22%, while in CAVM, it is at 13% of normal (figure, G).

We conclude that CAVM is allelic to XMEA, caused by a severe *VMA21* mutation. The CAVM mutation, c.164–6t>g, is one nucleotide over from a common XMEA mutation, c.164–7t>g.^{2,6} The latter eliminates the penultimate pyrimidine while c.164–6t>g removes the last pyrimidine in the polypyrimidine tract (figure, C and D). Tract abrogation is known to reduce splicing efficiency much more than tract interruption,⁷ consistent with the greater *VMA21* reduction by c.164–6t>g than by c.164–7t>g.

XMEA pathogenesis begins with reduced V-ATPases resulting in increased lysosomal pH, which hinders autophagosomes from digesting their contents. A feedback upregulation of autophagosome formation follows. The proliferating autophagosomes, unable to complete their autophagic cycles, become numerous debris-filled vacuoles, which merge to form the disease-pathognomonic large autophagic vacuoles.²

(A) Current photograph of the older of the 2 patients. (B) Electron micrograph from a muscle biopsy: large vacuoles with heterogeneous content/cellular debris within the myofiber and at the sarcolemma (arrowheads). Note the preserved sarcomeric structures around the former and exocytosis of contents by the latter between multiplied layers of basal lamina; bar, 2 μ m. (C) Diagram of the VMA21 gene. Boxes indicate exons. The sequence of the polypyrimidine tract upstream of the last exon is shown in full (double-underlined). The CAVM mutation is indicated as are all known XMEA mutations. (D) Electropherograms of control and mutated (CAVM) sequence show the thymine to guanine substitution in the patient with CAVM (arrows). (E) RT-PCR in lymphoblasts. For this and remaining panels, patients with CAVM in bold, patients with XMEA in regular font. (F) Quantitative RT-PCR in lymphoblasts as a ratio to the β -actin gene. Error bars in this and next panel represent SDs of 3 independent experiments; number of controls $n = 5$; control values were averaged and set as 1. (G) V-ATPase activity in lymphoblasts; number of controls $n = 5$; control values were averaged and set as 100%. CAVM = congenital autophagic vacuolar myopathy; RT-PCR = reverse transcription-PCR; V-ATPase = vacuolar-type H^+ -adenosine triphosphatase; XMEA = X-linked myopathy with excessive autophagy.

V-ATPases are present and crucial in all cell types, yet only skeletal muscle is affected, at least clinically. This remains the case in the patients with CAVM despite their profound loss of V-ATPase activity. Apart from mild cardiac hypertrophy, all organs, brain included, appear to function normally. Why only skeletal muscle is clinically affected in this disease remains elusive.

Our work shows that CAVM is allelic to XMEA and clarifies this disease prognosis over the first 20 years of life. Pathogenesis of CAVM/XMEA appears to be rooted in elevated lysosomal pH and autophagic dysregulation. Compounds that regulate lysosomal pH and autophagy could confirm this putative pathogenesis and could ultimately have a therapeutic role in converting patients with CAVM to the much more benign XMEA, and XMEA cases to health.

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