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Abnormal chaperone-mediated autophagy (CMA) in cardiomyocytes of a boy with Danon disease

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Abstract

Ultrastructural analysis of the cardiomyocyte structure in Danon disease reveals dramatic accumulation of abnormal late autophagic vacuoles (AVd) suggestive of primary lysosomal defect. Moreover, the accumulation of AVd in cardiomyocytes is consistent with a decreased rate of autophagic to lysosomal trafficking. These results suggest that the loss of the LAMP-2 protein strongly inhibits uptake of proteins into lysosomes for degeneration. The significant reduction of chaperone-mediated autophagy (CMA) activity in the affected cardiomyocytes induces a dramatic increase in the number and size of AVd and a severe reduction of myocardial contractility.

Key words: *Danon disease, chaperone-mediated autophagy, AVd.*

Introduction

Most mammalian cells have a specific intracellular system for degrading their own obsolete proteins or organelles. The major mechanism of degradation of cellular constituents is autophagy. Autophagy is a process necessary to maintain a well-controlled balance between anabolism and catabolism in order to have normal cell growth and development. This degeneration pathway allows the cell to eliminate unwanted or unnecessary organelles and recycle their components for reuse [7,9,17]. The role of autophagy as a cell repair and turnover mechanism is particularly important for long living post-mitotic cells such as cardiac myocytes, skeletal muscle cells and neurons, which are characterized by a very low (if any) replacement rate. Autophagy is involved in the most important cardiac pathologies including ischaemic heart disease and cardiomyopathies, a fact that has led to increased interest in this process [15]. Decreased autography or defect in completing autophagy results in accumulation of autophagosomes that may impair cell function. Malfunction of autophagic activity is suspected in Danon disease, in which excessive accumulation of autophagic vacuoles in skeletal muscle was described [1,5,10,11,12]. Because the most prominent morphological changes in Danon disease were described in skeletal muscle [1,11,13,14] we intended to show truncated chaperone-mediated autophagy (CMA) in a heart devoid of the LAMP-2 protein. In this ultrastructural study, we demonstrate abnormal autophagy in cardiomyocytes of a 19-year-old boy [8] with hypertrophic cardiomyopathy and LAMP-2 deficiency relentlessly leading to death at the age of 19.

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Material and methods

The endomyocardial biopsy taken from the apical septum of the right ventricle was analysed by electron microscopy. Heart specimens were fixed in 3% glutaraldehyde in phosphate buffer and post fixed in 1% osmium tetroxide in the same buffer. Then they were dehydrated and embedded in Spurr-resin. Thin sections double stained with uranyl acetate and lead citrate were examined with a JEMX/II electron microscope.

Results

Ultrastructural analysis of the structure of LAMP-2 deficient cardiomyocytes revealed dramatic accumulation of abnormal autophagic vacuoles suggesting a primary lysosomal defect. Various populations of autophagic vacuoles, sometimes very large, were observed in affected cardiomyocytes. One population of vacuoles contained morphologically intact sarcoplasmic compartments closely wrapped by 64 a double membrane (Fig. 1A-1B). These spherical vacuoles, sometimes very large (1 to 1.5 µm in diameter), were frequently located within the intramyofibrillar space. The morphology of these vacuoles was compatible with that of autophagosomes, also called early autophagic vacuoles (AVi). These structures are devoid of any lysosomal proteins. The second population of vacuoles, surrounded by a single membrane, contained partially degraded contents (Fig. 2). These structures, ranging in size from 2 to 2.3 µ, resembled late autophagic vacuoles (AVd). AVd were randomly distributed within myofibrillar spaces and were sometimes located in the perinuclear region. Membrane bound glycogen particles as well as free glycogen granules were found in all affected cardiomyocytes (Figs. 2, 3). The most in-

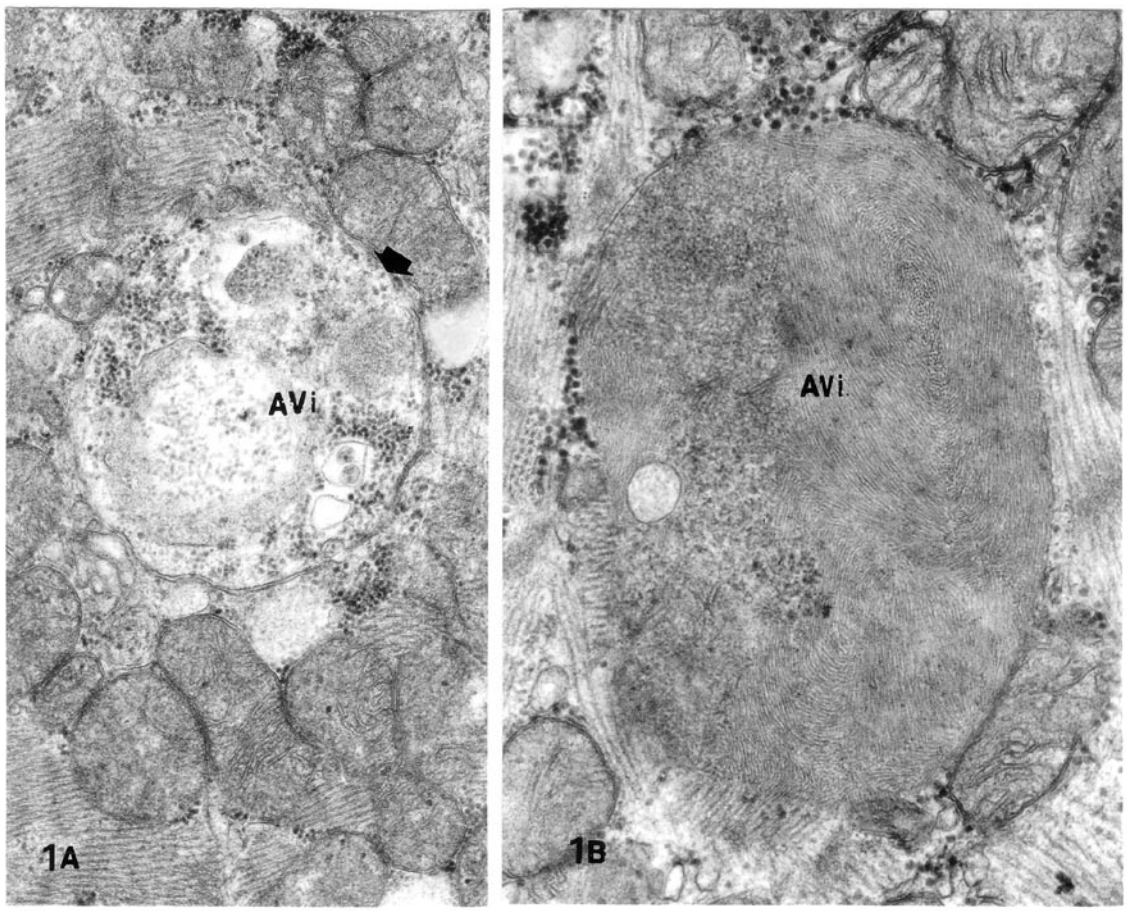

Fig. 1A-B. A. Early autophagic vacuole (AVi). Fragment of sarcoplasm enveloped by a double membrane (arrowhead). × 23 000. **B.** AVi containing filamentous material. × 30 000

triguing finding was the appearance of "hybrid" multivacuolar structures. They were present as very large, single membrane enveloped structures with two or more vacuoles visualized at their side (Figs. 3, 4). In addition large clusters of AVd vacuoles were observed in close contact with each other (Fig. 5) and sometimes a fusion "neck" was seen between two vacuoles (Fig. 6), suggesting that fusion occurred between AVd vacuoles. Occasional dense giant spherical lysosomes of 33-35 nm in diameter with an amorphous electron-dense matrix were found in the perinuclear region (Fig. 7). Contact between AVd and lysosomes in LAMP-2 deficient cardiomyocytes was very rarely observed. Autophagic vacuoles with basal lamina on the luminal side, frequently seen in the skeletal muscle, were not found in the affected heart.

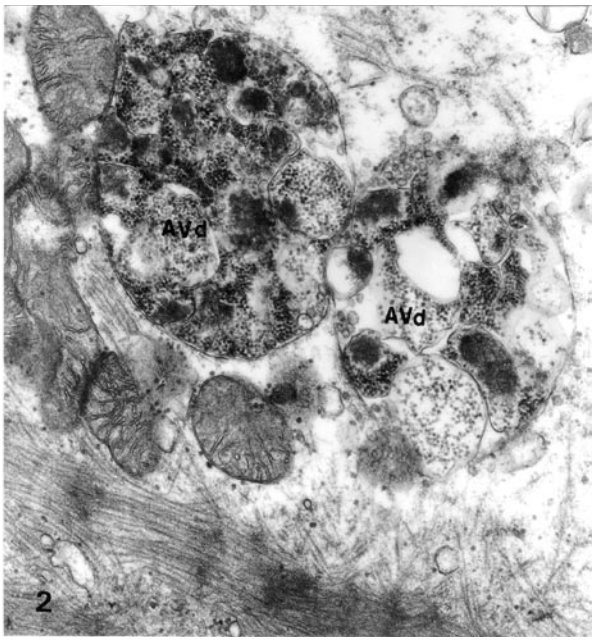

Fig. 2. Late autophagic vacuoles (AVd) surrounded by a single membrane. × 23 000

Discussion

The ultrastructural features of cardiocytes with LAMP-2 protein deficiency presented here indicate that the normal autophagic pathway is severely

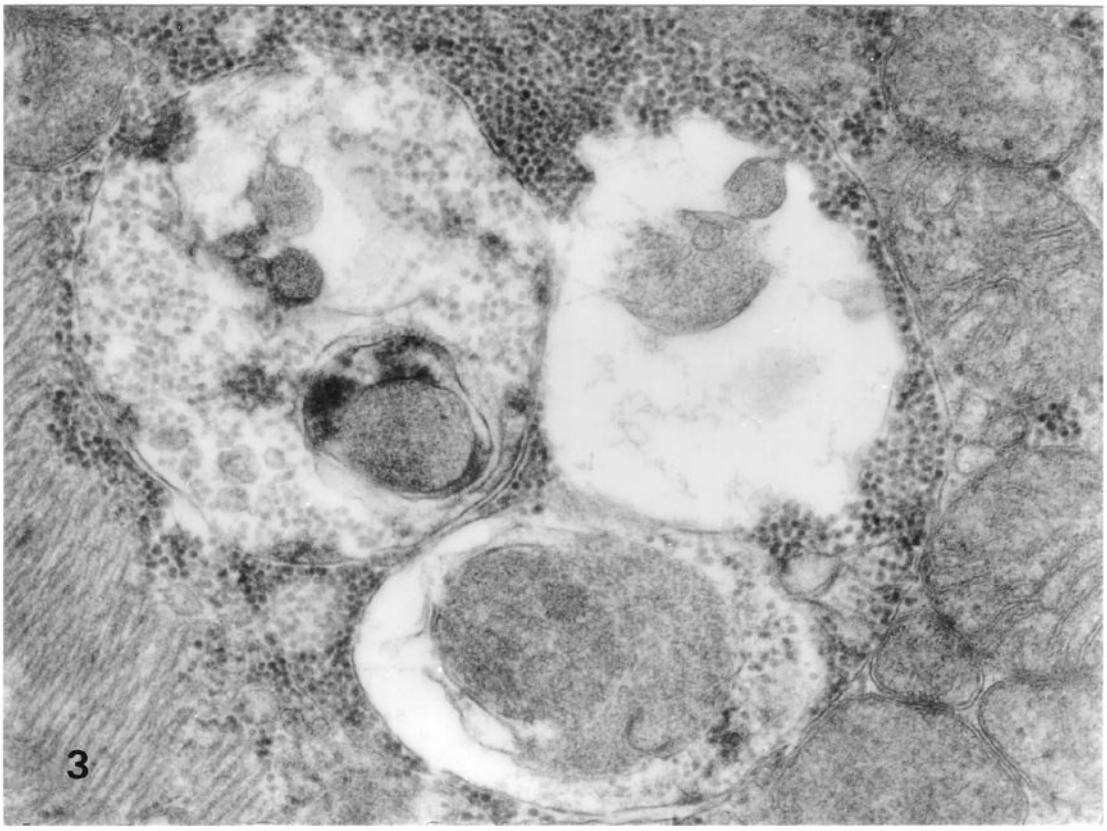

Fig. 3. "Hybrid" multivacuolar structure containing membrane enveloped vacuoles visualized at its side. × 34 000

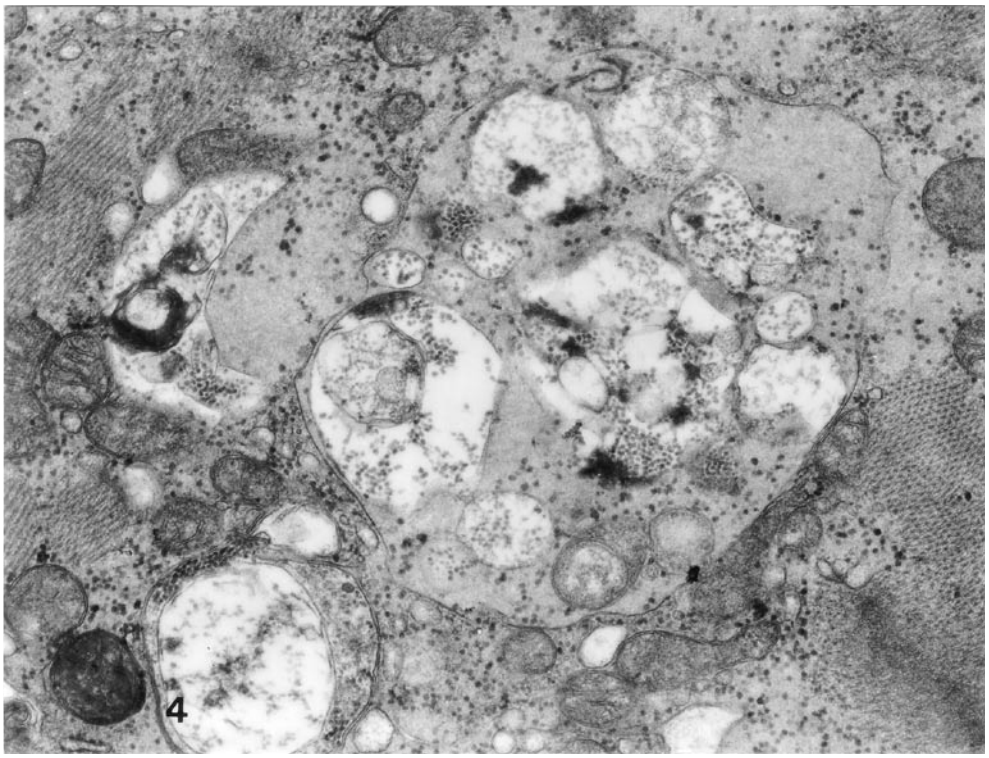

Fig. 4. A giant multivacuolar "hybrid". × 23 000

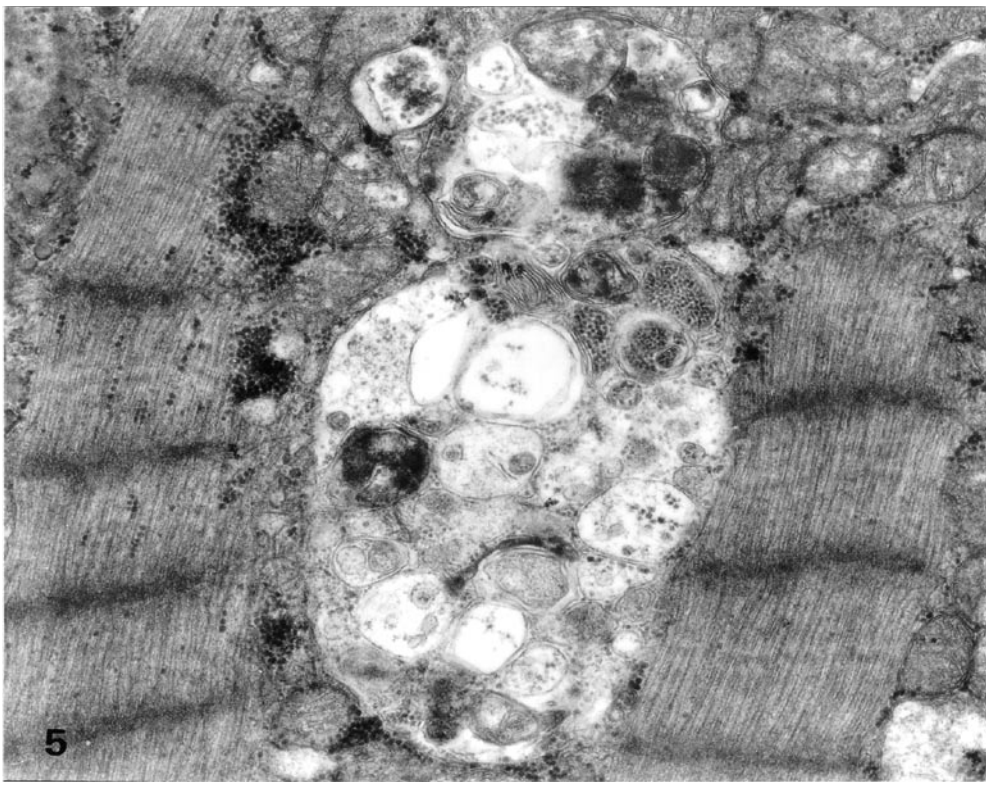

Fig. 5. AVd vacuoles in close contact with each other. × 23 000

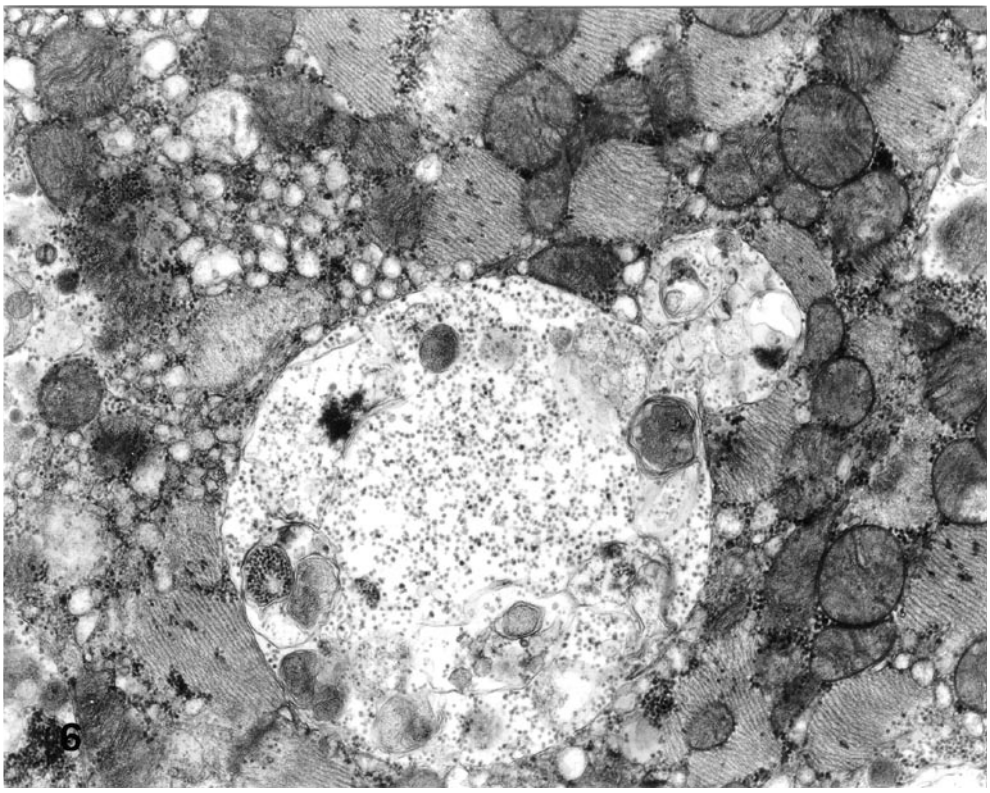

Fig. 6. A fusion "neck" seen between two vacuoles. × 15 000

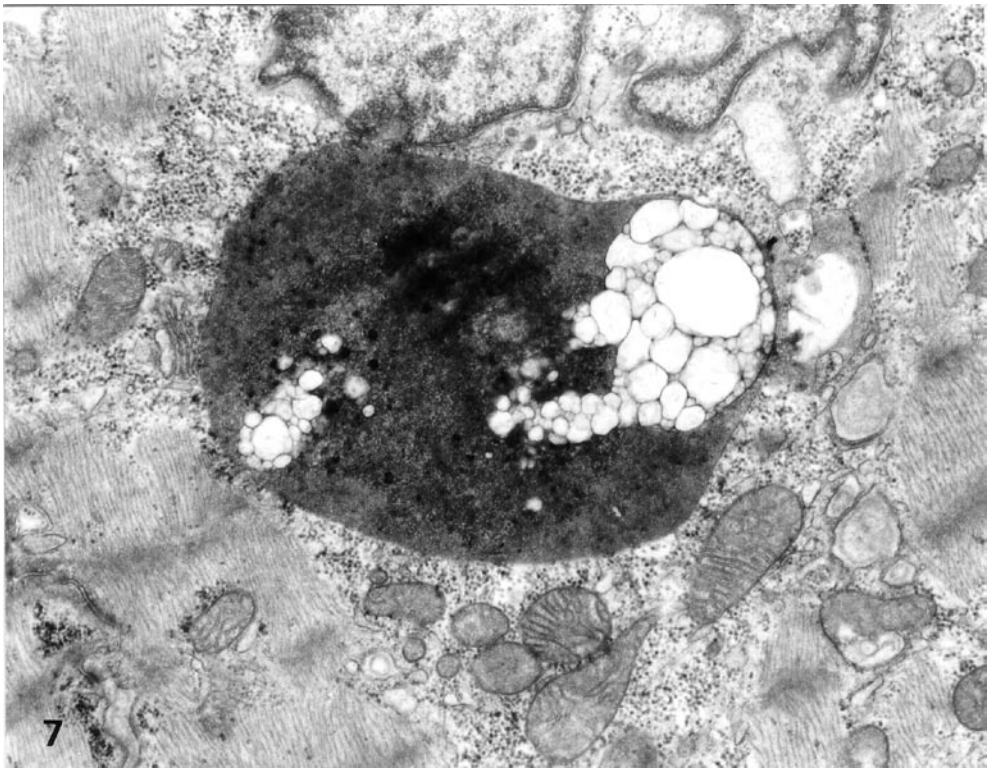

Fig. 7. A giant spherical lysosome with an amorphous electron-dense matrix. × 15 000

disturbed. Massive accumulation of AVd, the appearance of autophagic "hybrids", giant clusters of vacuoles as well as a significant decrease in the number of lysosomes may suggest that the ability of AVd to fuse with lysosomes is severely impaired. The defect in the LAMP-2 protein prevents digestion of unwanted or unnecessary organelles, resulting in accumulation of abnormal immature autophagic vacuoles within cardiomyocytes. It is highly plausible that cardiomyocytes with such severe degenerative vacuolar changes cannot generate contractile power and thus contribute to worsening of myocardiocyte function. In the classic process of autophagy, AVd mature rapidly by fusion with lysosomes [9,17] and acquire the lysosomal enzymes needed for the degeneration of an unnecessary material. The data presented above indicate a severe abnormality in the process of maturation of AVd to lysosomes. Most mammalian cells have a specific intracellular system for degrading their own obsolete proteins or organelles. In the heart and kidney, but not in the brain and skeletal muscle, chaperone-mediated autophagy (CMA) is a selective mechanism for the degradation of soluble cytosolic proteins in lysosomes [3,6,16]. CMA differs from normal macroautophagy in the mechanism by which substrates are delivered to lysosomes [4,6]. Substrate proteins are selectively targeted to lysosomes after interacting with the cytosolic chaperone hsc70/member of the 70kDA family of heat shock proteins [2]. This substrate-chaperone complex is targeted to the lysosomal surface, where it binds to the cytosolic tail of LAMP-2 at the lysosomal membrane before its translocation across the membrane into the lysosomal lumen and degradation within the lysosome matrix [3,4]. LAMP-2 is a highly N-glucosylated lysosomal membrane protein involved both in the fusion of autosomes with other membranes and in the maturation of autophagic vacuoles [4,7]. LAMP-2 level at the lysosomal membrane directly correlates with the activity of the proteolytic pathway. There is a strong correlation between the LAMP-2 level at the lysosomal membrane and the activity of the chaperone-mediated autophagic pathway [4,7]. These data indicate that LAMP-2 is involved in the process of fusion of autophagic vacuoles with lysosomes which provide acid hydrolases required for degradation, or has a function in the maturation of the autolysosomes into actively digesting organelles. The blockade of the substrate--LAMP-2 interaction in cardiomyocytes lacking this glycoprotein completely abolishes protein uptake. Loss of the LAMP-2 protein found in our case induces a dramatic increase in both number and size of AVi and AVd, suggestive of a block of transfer of the latter autophagic compartments to lysosomes.

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