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Pathology of skeletal muscle cells in adult-onset glycogenosis type II (Pompe disease): ultrastructural study

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Abstract

Ultrastructural analysis of the skeletal muscle cells in adult-onset Pompe disease revealed lysosomal and cytoplasmic glycogen storage, autophagic vacuoles and abnormal mitochondria. Significant glycogen accumulation within lysosomes causes their rupture and release of glycogen into the cytoplasm. Excess cytoplasmic glycogen could lead to damage of the structure of muscle cells including myofibrils. In consequence, parts of the sarcoplasm and damaged organelles were sequestered within membrane-limited vacuoles. We suppose that massive accumulation of autophagic vacuoles results from the inability of destroyed lysosomes to fuse with vacuoles. Autophagic vacuoles may be a prominent feature of muscle cells in adult glycogenosis type II.

Key words: adult-onset Pompe disease, skeletal muscle cells, ultrastructure, lysosomes, autophagic vacuoles.

Introduction

Pompe disease (type II glycogenosis) is an autosomal recessive disorder caused by genetic defects in lysosomal acid α-glucosidase (GAA) leading to the accumulation of glycogen in lysosomes of multiple tissues including skeletal muscle. The severity of clinical manifestations of Pompe disease depends on the nature of the genetic abnormality and the amount of enzyme activity [10]. Partial enzyme deficiency is noted in juvenile or adult patients [11]. In adult patients the pathology is usually present in skeletal muscle with respiratory muscle involvement [7]. On the other hand, GAA deficiency *in vitro* in late-onset type II glycogenosis was not directly proportional to the amount of glycogen storage and disease severity [16]. At the ultrastructural level, the skeletal muscle cells revealed accumulations of glycogen granules and various degenerative changes including a loss of normal sarcomere structures and vacuolar degeneration [1,14,15]. Here, we report the ultrastructural examination of skeletal muscle biopsies from patients with adult-onset Pompe disease.

Material and Methods

The study was performed on biopsy material derived from 3 patients with adult-onset Pompe disease. Basic

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Table I. Clinical, ultrastructural and biochemical data in patients with type II glycogenesis (late-onset)

Patient	Onset of disease	The first symptome	Breathlessness	Course of the disease	Electron microscopy	GAA (norm 0.36)
1st (19 years old)	18 years old	weakness	+++++	acute	+++	0.07
2nd (31 years old)	25 years old	weakness	++	subacute	++	0.07
3rd (29 years old)	27 years old	weakness	+	mild	+	0.09

clinical, ultrastructural and biochemical data of the patients are presented in Table I. Detailed clinical data have been presented in our previous paper [16].

Biopsies were performed on the biceps muscle in 3 patients. Biopsy specimens were fixed in 2.5% glutaraldehyde and postfixed with 2% osmium tetroxide. Semi-thin sections stained with toluidine blue were examined with light microscopy. Ultrastructural analysis was carried out on ultrathin sections of muscles after staining with uranyl acetate and lead citrate in a transmission electron microscope Opton DPS 109.

Results

In three analyzed biopsies of skeletal muscle, ultrastructural examination revealed pathological features but with different intensity and in various number of fibres for each patient. Similar intensity of ultrastructural pathology of the muscles was exhibited

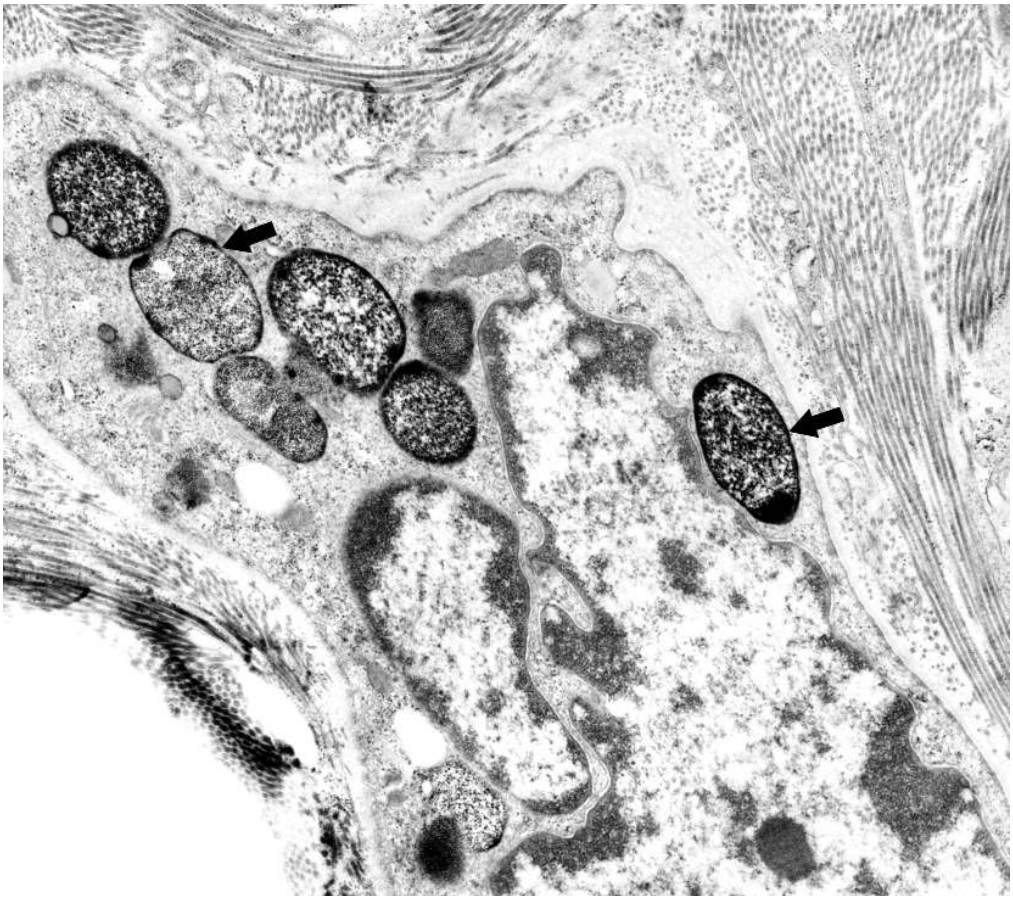

Fig. 1. Numerous lysosomes (arrows) in sarcoplasm of muscle cell. Patient 1, original magnification × 7000

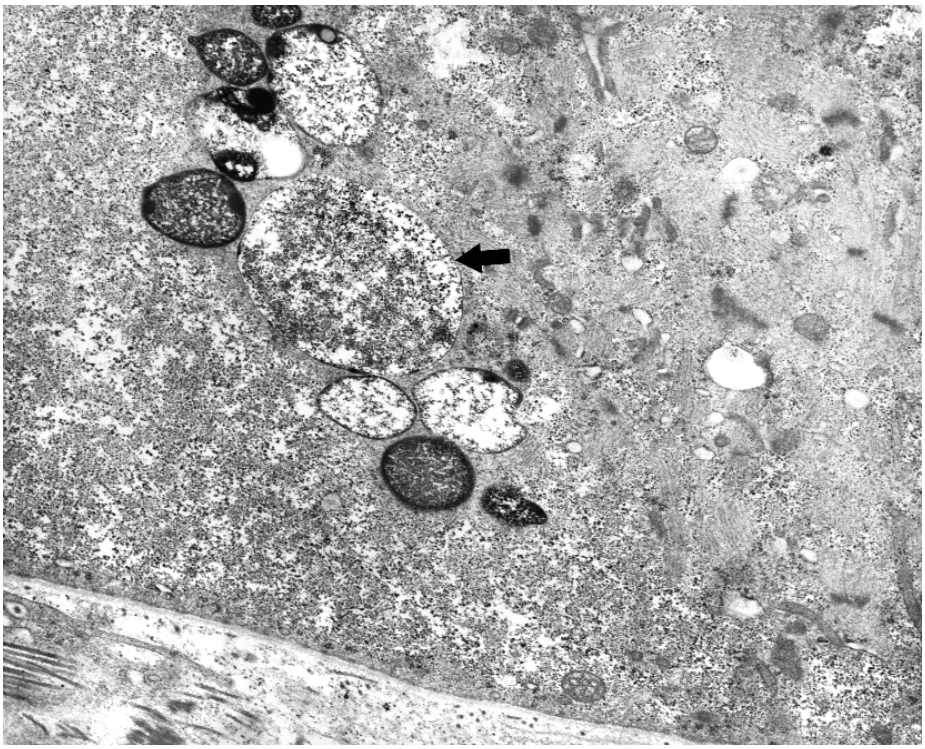

Fig. 2. Glycogen-filled lysosomes of various size and morphology within destroyed myofilaments. A giant lysosome (arrow). Patient 1, original magnification × 7000

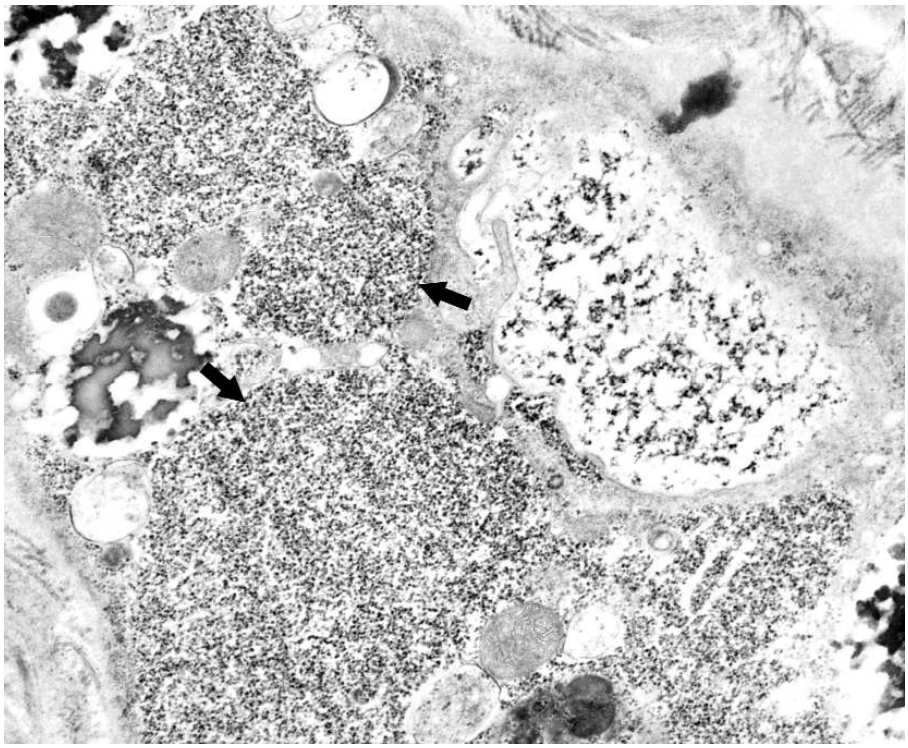

Fig. 3. Giant glycogen-filled lysosomes (arrows) located under sarcolemma. Patient 1, original magnification × 7000

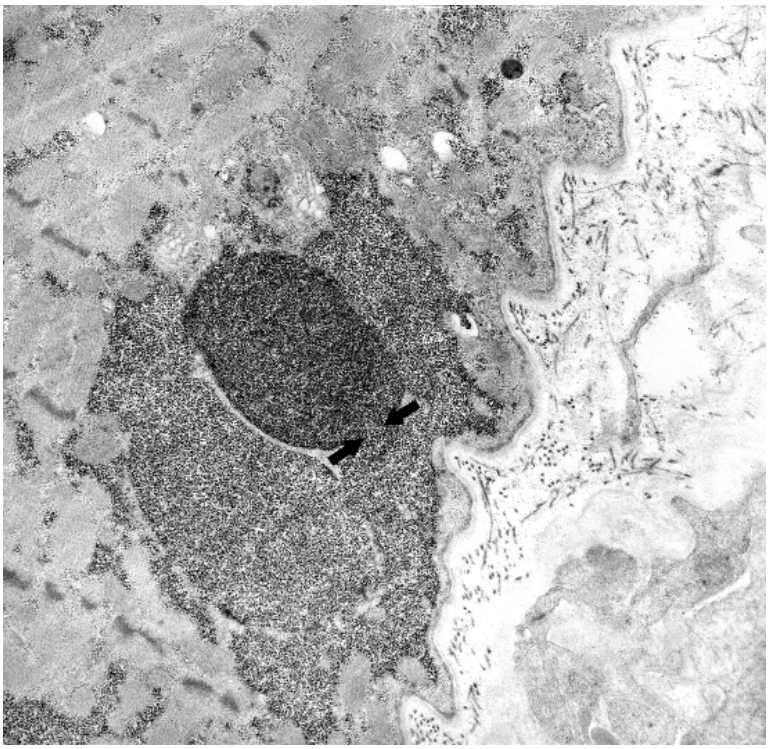

Fig. 4. Lysosome with ruptured membrane (arrows) and release of glycogen into cytoplasm. Patient 1, original magnification × 7000

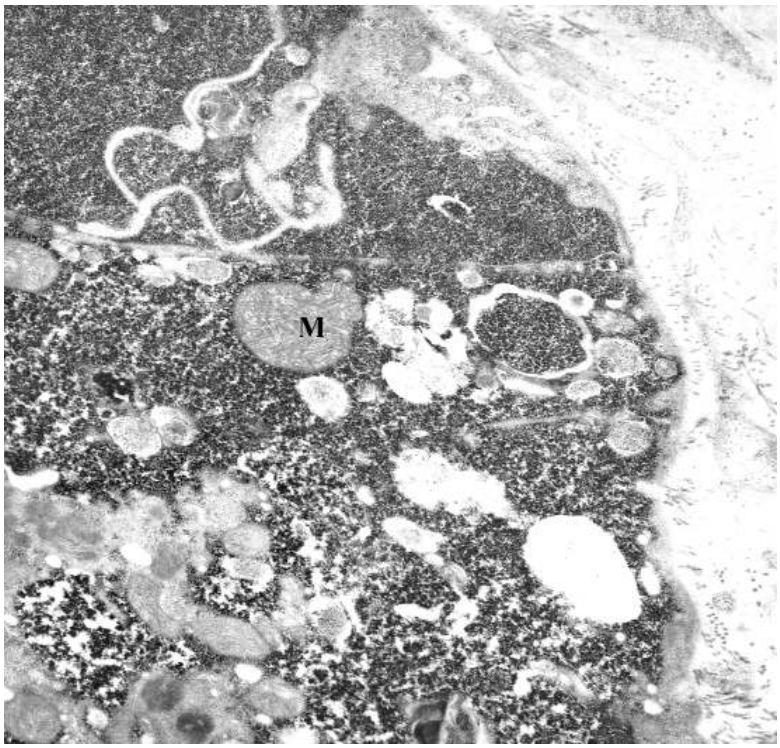

Fig. 5. Massive accumulation of free glycogen and vacuoles filling most of cell, mitochondrium (M). Patient 1, original magnification × 7000

in patients 1 and 2, but in patient 1 the normal ultrastructure was almost completely destroyed in most muscle fibres. The muscle cells displayed numerous glycogen-filled lysosomes (Figs. 1-3). These lysosomes were of various size and morphology. Some of them showed heterogenic morphology and/or giant size (Figs. 2, 3). Sometimes lysosomal rupture and release of glycogen granules into the cytoplasm were visible (Fig. 4). Cytoplasmic glycogen usually occupied a large area of affected muscle cells, which were devoid of many myofilaments and sarcomeres (Figs. 5, 6). Massive accumulation of vacuoles was also visible (Figs. 7, 8). The morphology of these vacuoles resembled autophagic vacuoles. Autophagic vacuoles revealed various size and content including glycogen granules and mitochondria (Fig. 8). An enormous number of autophagic vacuoles were surrounded by a single membrane like late autophagic vacuoles (Figs. 7, 8). Additionally, multivacuolar and multilamellar structures were found in the sarcoplasm (Figs. 9, 10). The most damaged muscle fibres contained numerous mitochondria that were abnormal in size and morphology. Numerous mitochondria with paracrystalline inclusions were visible in extensive destroyed fibres of patient 2. Such mitochondria were located in areas of sarcoplasm containing vacuoles, glycogen-filled lysosomes as well as free glycogen granules and autophagic vacuoles (Figs. 11, 12). In patient 3, among many relatively normal muscle fibres there were only a few destroyed fibres. Typically, muscle cells in this patient showed few and small size glycogen-filled lysosomes (Figs. 13, 14). Only a few free glycogen particles as well as vacuoles were present, but mitochondria were multiple (Fig. 14).

Discussion

In our previous paper, we concluded that the amount of glycogen storage vacuolar degeneration and disease severity in late-onset type II glycogenosis were not directly proportional to GAA activity in vitro [16]. In the present study, with electron microscopy we found both intralysosomal and free cytoplasmic glycogen as well as various autophagic vacuoles in the skeletal muscle of examined patients. The most intensive pathological changes were found in patients 1 and 2. The majority of their muscle cells showed numerous and very enlarged lysosomes accompanied by massive accumulation of free

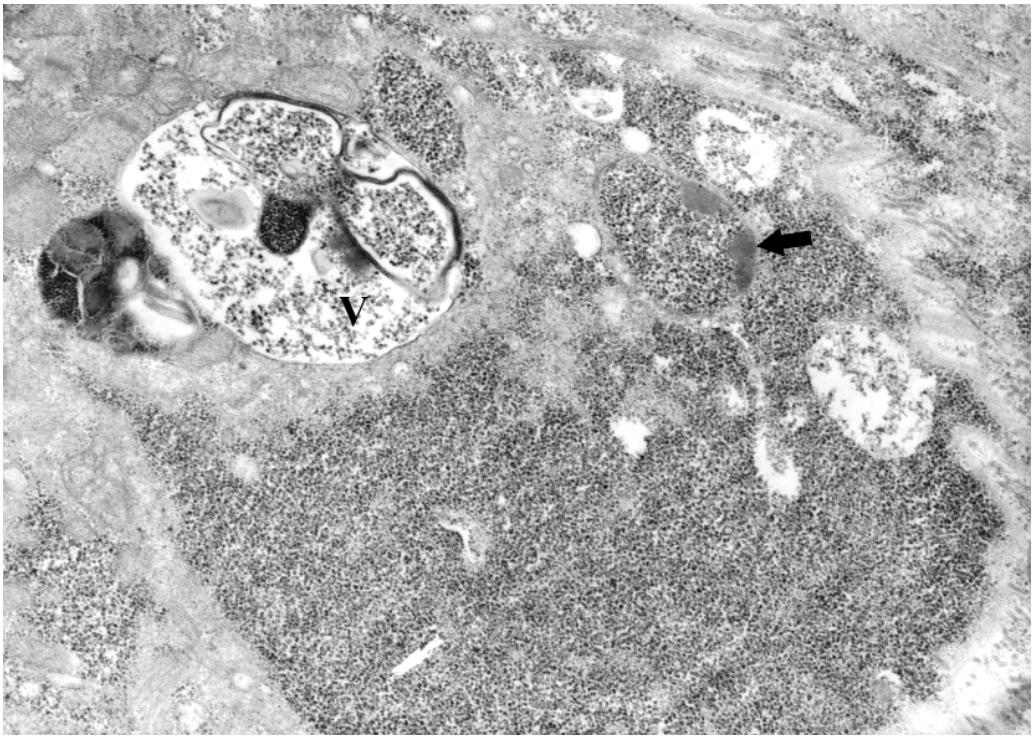

Fig. 6. Free glycogen, vacuoles (V) and lysosomes (arrow) under sarcolemma. Patient 1, original magnification × 7000

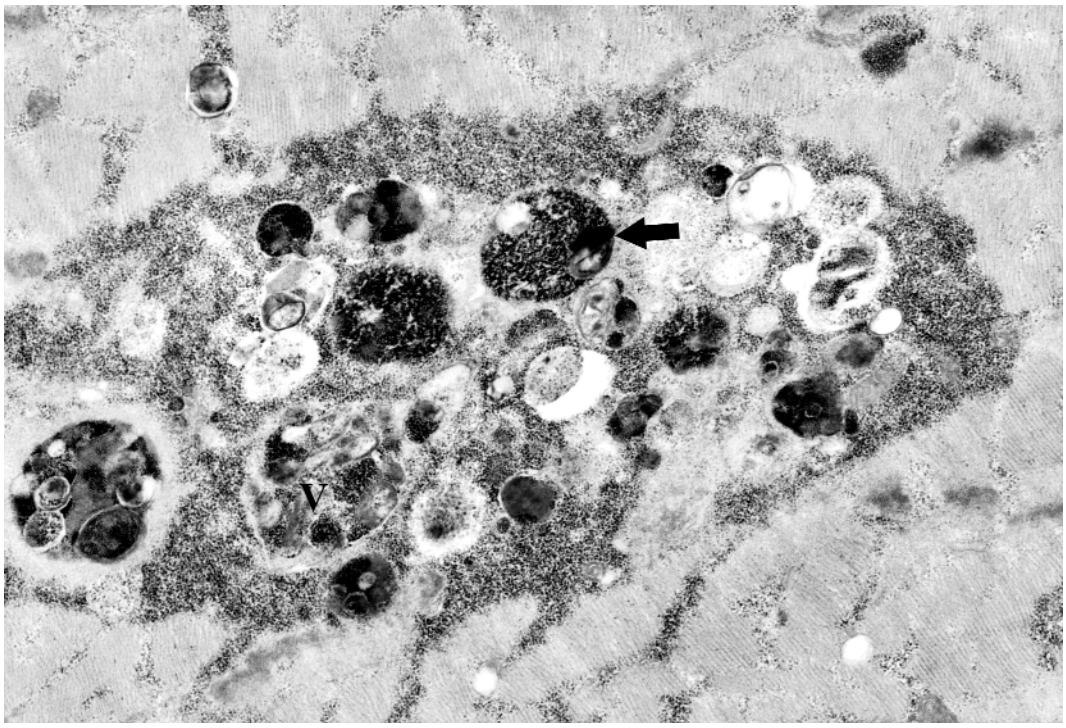

Fig. 7. Numerous vacuoles (V) and lysosomes (arrow) located within intramyofibrillar space. Patient 1, original magnification × 7000

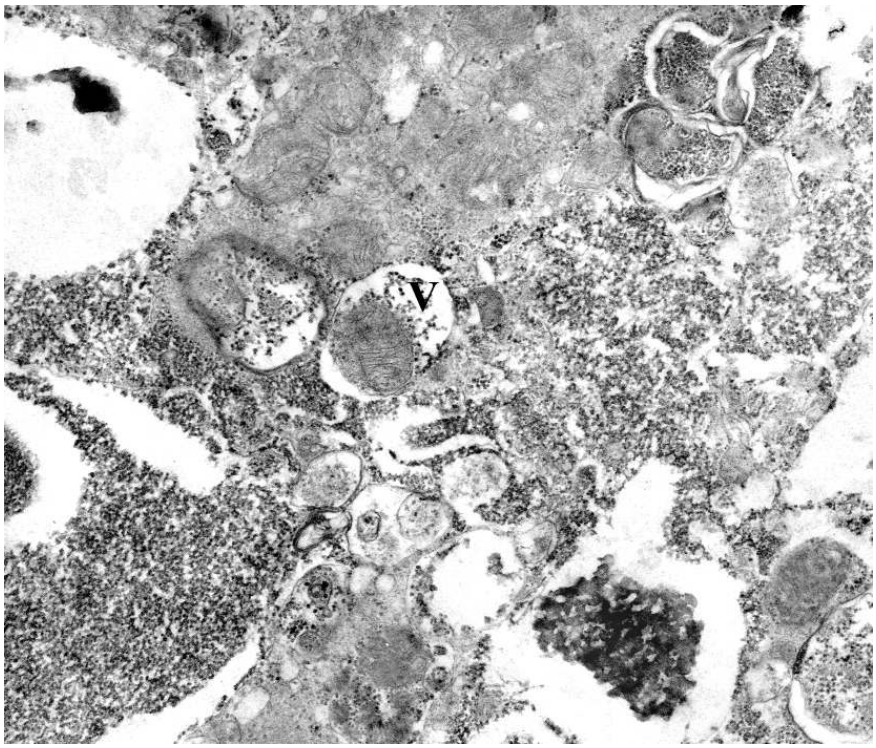

Fig. 8. Autophagic vacuoles with various content. Vacuole containing mitochondrion (V). Patient 1, original magnification × 12 000

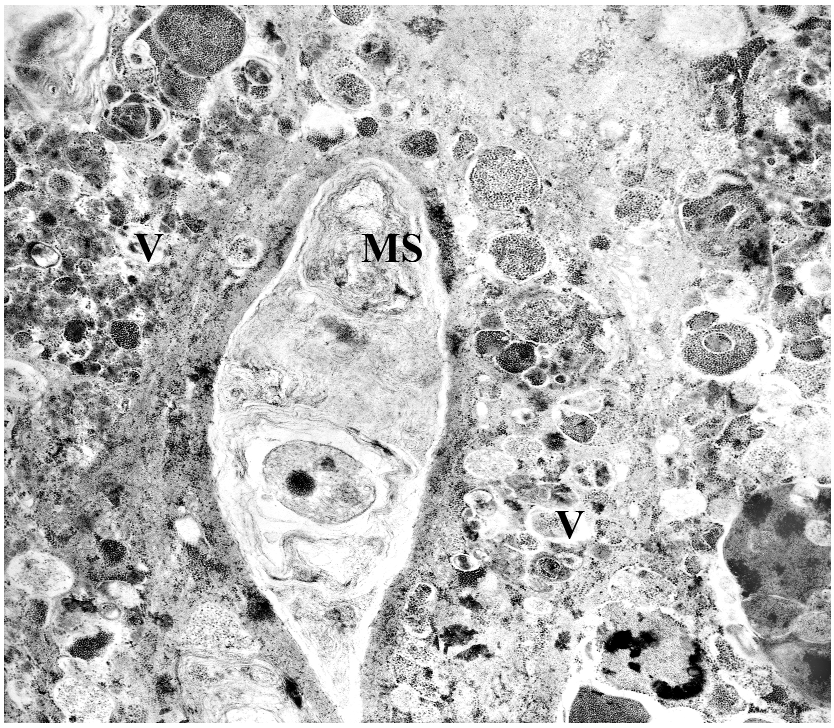

Fig. 9. Multilamellar structure (MS) in area with numerous vacuoles (V). Patient 2, original magnification × 7000

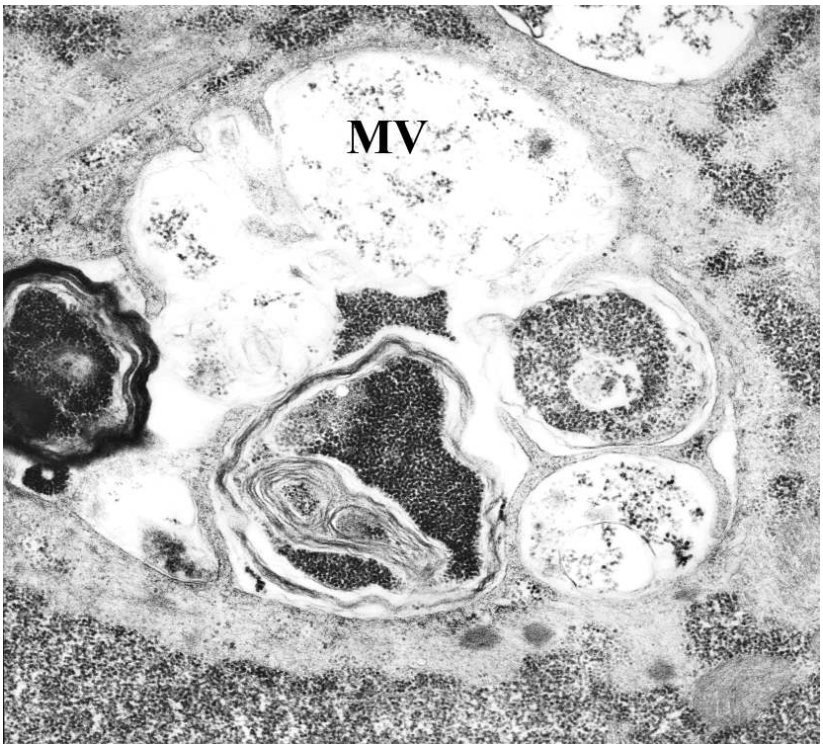

Fig. 10. Big multivacuolar and multilamellar structure (MV) in area of muscle cell with cytoplasmic glycogen. Patient 1, original magnification × 12 000

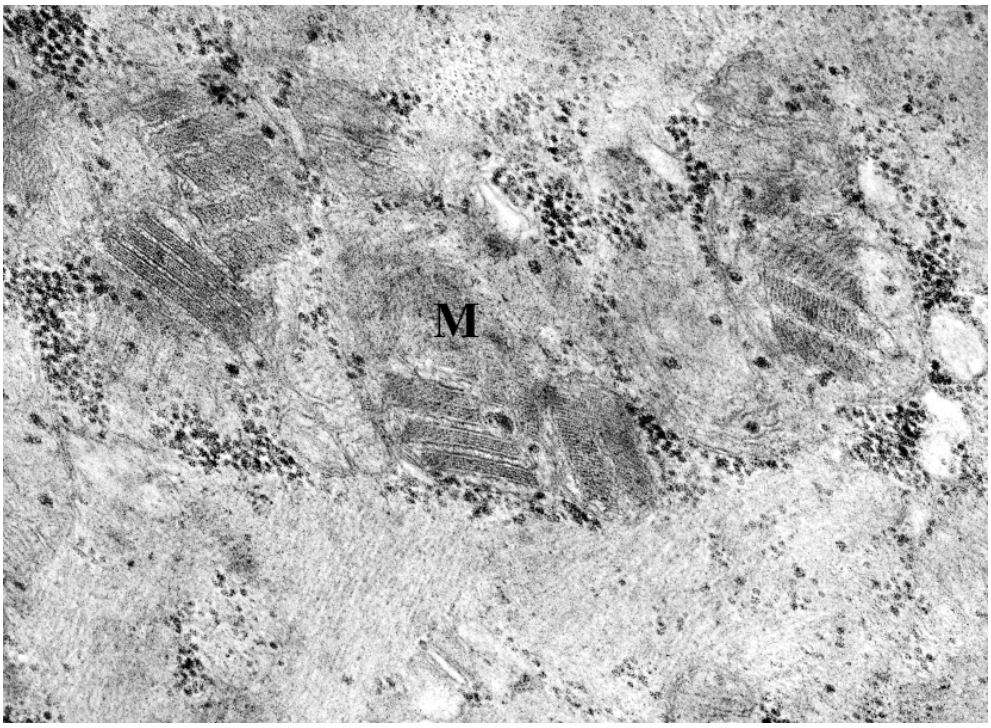

Fig. 11. Mitochondria with paracrystalline inclusions (M) located within intramyofibrillar space. Patient 2, original magnification × 20 000

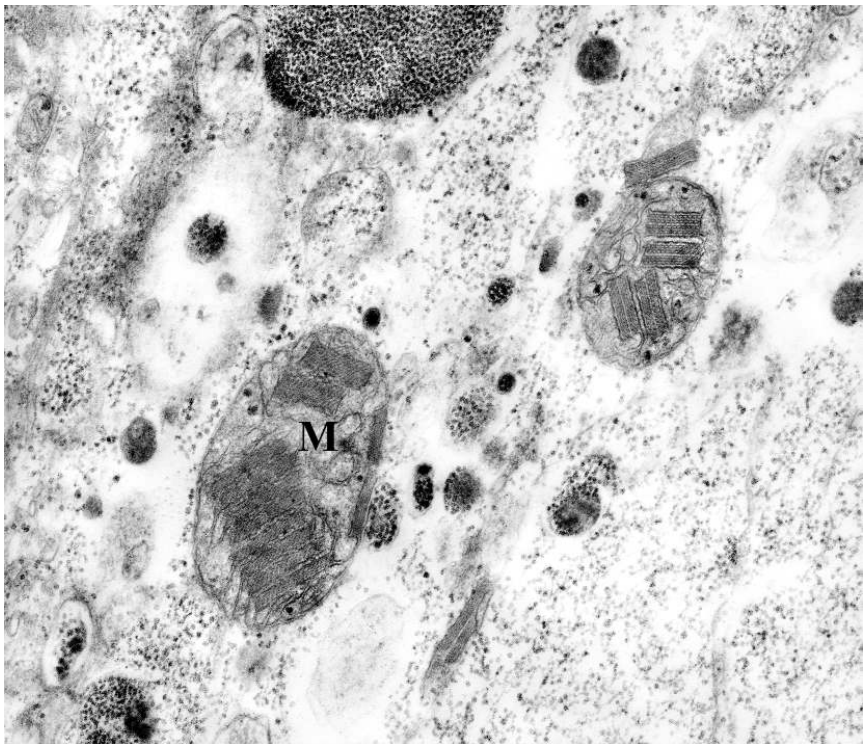

Fig. 12. Mitochondria with paracrystalline inclusions (M) in swollen area of muscle cell. Patient 2, original magnification × 20 000

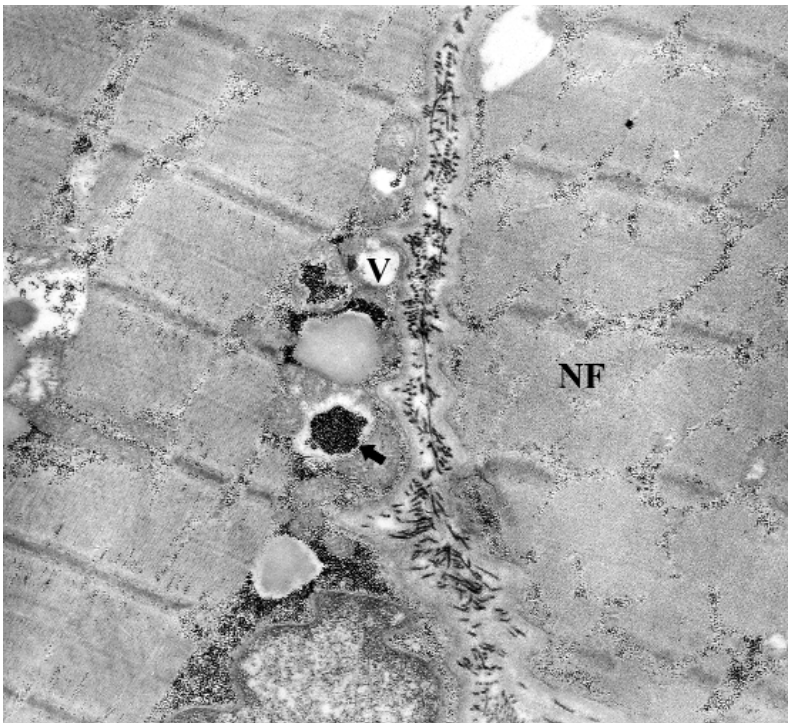

Fig. 13. Fiber with glycogen-filled lysosome (arrow), free glycogen and vacuoles (V) under sarcolemma. Fiber with normal morphology (NF). Patient 3, original magnification × 7000

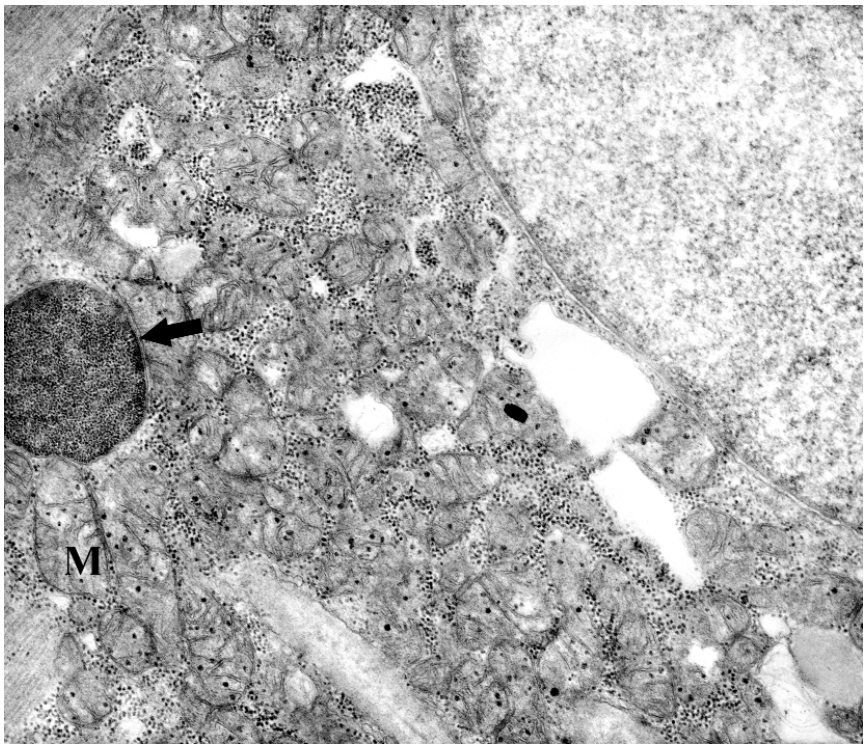

Fig. 14. Numerous abnormal mitochondria (M) and glycogen-filled lysosome (arrow). Patient 3, original magnification × 12 000

glycogen in the sarcoplasm. These fibres exhibited clusters of autophagic vacuoles, loss of myofilaments and multiple mitochondria with changed morphology. Similarly to our findings a progressive autophagic build-up in addition to enlargement of glycogen-filled lysosomes in muscle have previously been shown in both the patients and the animal model of Pompe disease [3,8,10,16]. The accretion of autophagic vacuoles may be a prominent feature in older patients [13].

In accordance with earlier ultrastructural examinations of skeletal muscle cells from infantile Pompe disease [6,15] we assume that giant lysosomes with ruptured membranes release glycogen into the cytoplasm, leading to cytoplasmic glycogen accumulation in muscle in late-onset disease. Massive intracellular glycogen accumulation causes secondary severe pathology of muscles. These muscle cells revealed damage and loss of myofilaments as well as various vacuoles. The largest autophagic vacuoles were visible in numerous skeletal fibres with a large area of free glycogen particles in the cytoplasm of patients 1 and 2. The autophagic areas occupied more than half of the diameter of the fibre, but sometimes the vacuoles filled almost all muscle fibres. An increase in autophagy in Pompe skeletal muscle may be coupled with the inability of defected lysosomes to fuse with autophagic vacuoles. The cause of autophagy might be nutrient limitation [4] and/or oxidative stress [9]. The autophagy might be up-regulated to remove the damaged lysosomes [3]. However, the autophagosome formation are probably generated from defective cytoplasmic constituents including organelles such as mitochondria observed here in vacuoles. In our patients, most autophagic vacuoles showed only a single limited membrane like in late autophagic vacuoles. These data indicate abnormality in the process of fusion with disturbed lysosomes as was postulated by others [2]. Massive accumulation of autophagic vacuoles and loss of myofilaments could be responsible for dysfunction of muscle including muscle weakness in patient with adult-onset form of glycogenosis type II.

Based on the ultrastructural pictures of skeletal muscle from Pompe patients it is postulated that as a consequence of the release of lysosomal glycogen into the cytoplasm, the mitochondrial structure becomes abnormal [5,15]. Similar alterations of mitochondria that concerned the number, size, shape and morphology were found in more damaged muscle cells of our two patients. The most characteristic mitochondrial abnormalities in patients 1 and 2 were paracrystalline inclusions. Some abnormally shaped mitochondria contained concentric and irregular cristae. It is suggested that mitochondria may be mildly reduced in Pompe disease, because massive accumulation of undigested glycogen causes ultrastructural distortions, which lead to disruption of overall cell function [12].

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