

Adult glycogenosis type II (Pompe's disease): morphological abnormalities in muscle and skin biopsies compared with acid alpha-glucosidase activity

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Abstract

Glycogen storage disease type II (GSD II) is an autosomal recessive deficiency of acid alpha-1,4-glucosidase (GAA) caused by mutations in the GAA gene located on human chromosome 17 (17q 25.2-q 25.3). Although its pathophysiology is partially understood, it has not yet been elucidated whether the level of GAA deficiency is directly proportional to the level of glycogen storage, vacuolar degeneration and/or GSD II severity.

Muscle and skin biopsies were taken from three female patients with symptoms of progressive muscle weakness and respiratory failure: patient 1 aged 19, as well as patients 2 and 3 (two sisters) aged 31 and 29, respectively. Initial clinical manifestations, respiratory failure and muscle weakness, were similar in all the examined patients, while their character and intensity differed.

For each examined patient, the activity of lysosomal GAA (at pH 3.8) was measured fluorometrically in isolated blood leukocytes (L) and dried blood spots (DBS). Biopsy samples were studied histologically, immunohistologically and ultrastructurally. Each of them displayed similar morphological features, although with different intensity.

Muscle fibres were irregular in size with smaller non-rounded fibres and vacuolar degeneration. In vacuoles, we observed glycogen and intense positive ubiquitin reaction. Myofibrils were almost completely destroyed by the accumulation of glycogen granules in lysosomes and those free, without limiting membranes as well as by vacuoles of various size. Autophagic vacuoles were visible occasionally. Excess glycogen was also present in the walls of muscle and skin capillaries.

All three patients showed reduced GAA activity ratios measured at pH 3.8 with and without acarbose (patient 1 – 0.12 in DBS and 0.07 in L; patient 2 – 0.05 in DBS and 0.07 in L; and patient 3 – 0.12 in DBS and 0.09 in L).

Based on the study results, we concluded that GAA deficiency in vitro in late-onset type II glycogenosis was not directly proportional to the amount of glycogen storage, vacuolar degeneration and disease severity.

Key words: adult glycogenosis type II, histochemistry, ultrastructure, GAA activity

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Introduction

Glycogen storage disease type II (GSD II) is an autosomal recessive lysosomal disorder, caused by deficiency of acid alpha-1,4-glucosidase (GAA). Deficiency of GAA can be caused by about 150 mutations found in the GAA gene located on human chromosome 17 (17q25.2-q25.3) [6].

This storage disease was first reported by Pompe and Putschar in 1932 [14]. Following the identification of lysosomes, it was found out that Pompe's disease occurs due to the absence of glycogen degrading lysosomal enzyme GAA. Excess glycogen is present in cardiac muscle fibres, smooth and skeletal muscles, as well as in vascular endothelium, Schwann cells, perineurium and astrocytes in the cerebral cortex [8,13,14,18]. In the infantile and juvenile forms of GSD II, the storage of glycogen is also visible in neurons in anterior horn cells, motor cranial nerve nuclei, basal ganglia and gastrointestinal tract plexuses [7]. Therefore, intra-lysosomal accumulation of glycogen is present in almost all body tissues [6].

Three forms of this storage disease (infantile, juvenile and adult onset types) have been described based on its severity and the age that onset of clinical symptoms is observed. It is supposed that the severity of the disease depends on the degree of GAA deficiency [5,14].

Material and Methods

Biopsies were performed on the biceps muscle and skin in each patient. Biopsy specimens of muscle and skin were fixed in 4% paraformaldehyde in 0.1 M phosphorane buffer saline, pH 7.4 for light microscope examination, and in 2.5% glutaraldehyde and postfixed in 2% osmium tetroxide for ultrastructure analysis. All biopsy samples were stained histologically (haematoxylin-eosin, PAS, PAS-dimedon), and immunohistologically with ubiquitin (DAKO 1:200).

Ultrastructural analysis was carried out on ultrathin sections of skin and muscles after staining with uranyl acetate and lead citrate, and examined with a transmission electron microscope (Opton DPS109).

Lysosomal acid alpha-glucosidase activity was measured fluorometrically in isolated peripheral blood leukocytes (L) and dried blood spots (DBS) with 4-methylumbelliferyl-alpha-D-glucopyranoside (4MU-alphaGlc) as substrate according to previously described methods with slight modifications [2,10].

Maltase-glucoamylase activity was inhibited with 30 $\mu\text{mol/L}$ acarbose. For each patient, GAA activity was assayed in the presence and absence of acarbose at pH3.8. The results are expressed as the ratio of GAA activities with and without acarbose.

Clinical data/case reports

Patient 1

A 19-year-old woman was admitted to hospital because of rapid progressive respiratory failure. Her first symptoms began 7 months before admission. Initially, she complained about occasional exercise dyspnoea occurring during running and walking. Within two months exercise dyspnoea progressed and occasional resting dyspnoea occurred. The symptoms were accompanied by severe morning headaches and progressive muscle weakness. Due to menstrual cycle disturbances, the patient was referred to the endocrinological department, where hyperprolactinaemia and hypothyroidism were diagnosed. During hospitalisation, the patient was consulted neurologically and subjected to MRI scanning, which revealed diffuse T2 weighted changes in the brain. After multiple sclerosis was diagnosed, the patient underwent steroid treatment, which failed to be effective. Symptoms dramatically progressed. Exercise dyspnoea on every effort, obstructive sleep apnoeas and severe morning headaches were present daily. The patient was bedridden and finally transferred to the emergency department with severe respiratory failure and acidosis. She was admitted to the intensive care unit, put on the respirator, and subjected to IVIg treatment (intravenous immunoglobulin in the dose of 1 g per kilogram of body weight on 5 consecutive days), which also appeared to be ineffective. The neurological examination revealed flaccid tetraparesis, proximal muscle atrophy and respiratory failure. Electromyography (EMG) showed myopathic changes. A muscle and skin biopsy was taken and acid maltase activity was determined and the diagnosis made.

Patient 2

A 29-year-old woman was admitted to the hospital because of progressive nocturnal dyspnoea, muscle weakness and occasional spontaneous dyspnoea occurring during the day for two months.

Symptoms were more pronounced in the evening and showed a fluctuating intensity. Her history revealed the diagnosis of juvenile myoclonic epilepsy successfully treated with valproates. She had been free of attacks for the last two years. On neurological examination she revealed no abnormalities. EMG showed mild myopathic changes present seen only in the brachial biceps muscle. The muscle biopsy and activity of acid maltase confirmed the diagnosis.

Patient 3

A 31-year-old woman, sister of patient 2, complained about muscle weakness, occasional dyspnoea, poor exercise tolerance and exercise dyspnoea for 6 years. She was diagnosed for hepatitis after laboratory findings indicating elevated levels of serum aminotransferases and creatinine kinase. The patient underwent hepatic biopsy, which revealed no significant changes. Symptoms had progressed during the last year. On neurological examination the patient revealed proximal muscle weakness, Gower's phenomenon, and lack of deep tendon reflexes from biceps brachii, as well as lack of the patellar reflex. EMG showed advanced myopathic changes in all tested muscles. The biopsy of brachial muscle biceps and activity of acid maltase confirmed the diagnosis.

Results

Alpha-glucosidase activity at pH 3.8 was assayed in the presence and absence of acarbose in DBS and L. The assay revealed its reduced activity in all three patients (Table I).

The ratio of GAA activities in leukocytes were reduced to 0.07 in patients 1 and 2, while in patient 3 it was 0.09; in DBS the ratio was reduced to 0.12 in patients 1 and 3, but to 0.05 in patient 2.

Each of the investigated muscle and skin specimens showed similar features, but with various intensity of changes. Muscle fibres were irregular in size with smaller non-rounded fibres and revealed intracellular vacuolar degeneration. These vacuoles of various size and shape with a strong demonstrated PAS, positive granular material and intensive ubiquitine activity (Fig. 1A-C). Vacuoles, irregularly shaped, showed similar grade of reaction with ubiquitine in the muscles of all the three patients (Fig. 2A-C). In patients 1 and 2, glycogen was abundant inside the muscles. The intensity of glycogen storage did not correspond with the degree of GAA deficiency and vacuolation. Glycogen accumulation was also present in the walls of capillaries of muscle and skin (Fig. 3A-C).

On electron microscope, the patients' biopsies showed analogous degenerative changes.

Table I. Alpha-glucosidase activity at pH 3.8 expressed as ratio \pm acarbose

| Patient | DBS | Leukocytes |
|----------------|--|--|
| | Mean \pm SD = = 0.38 \pm 0.05 (n=38) Range: 0.29-0.49 | Mean \pm SD = = 0.4 \pm 0.06 (n=9) Range: 0.31-0.52 |
| No. 1, aged 19 | 0.12 | 0.07 |
| No. 2, aged 31 | 0.05 | 0.07 |
| No. 3, aged 29 | 0.12 | 0.09 |

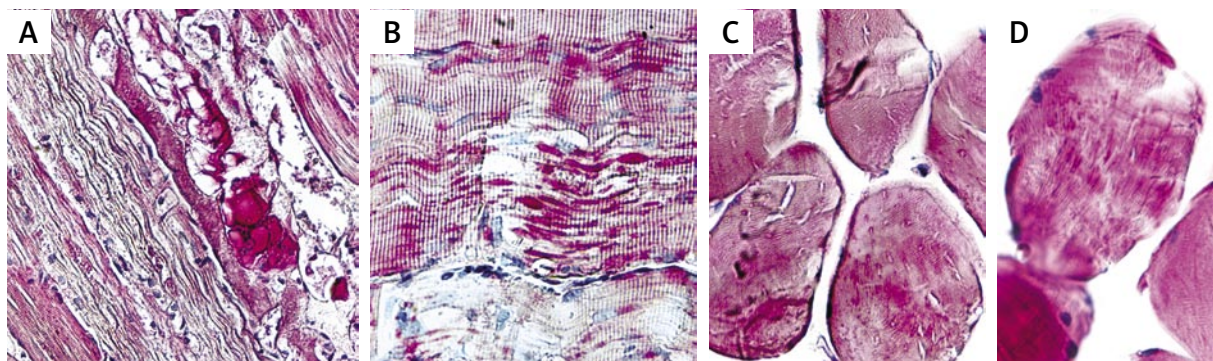


Fig. 1. Intensity of glycogen storage in muscle. PAS staining. (A) Patient 1 (\times 200); (B) Patient 2 (\times 200); (C, D) Patient 3 (\times 400)

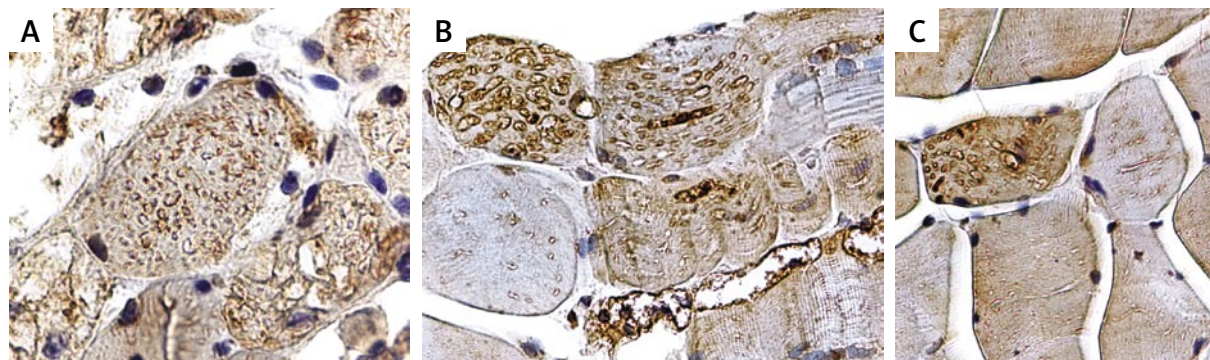


Fig. 2. Vacuolar degeneration of muscle fibres. Reaction with ubiquitine. (A) Patient 1 (× 630); (B) Patient 2 (× 400); (C) Patient 3 (× 400)

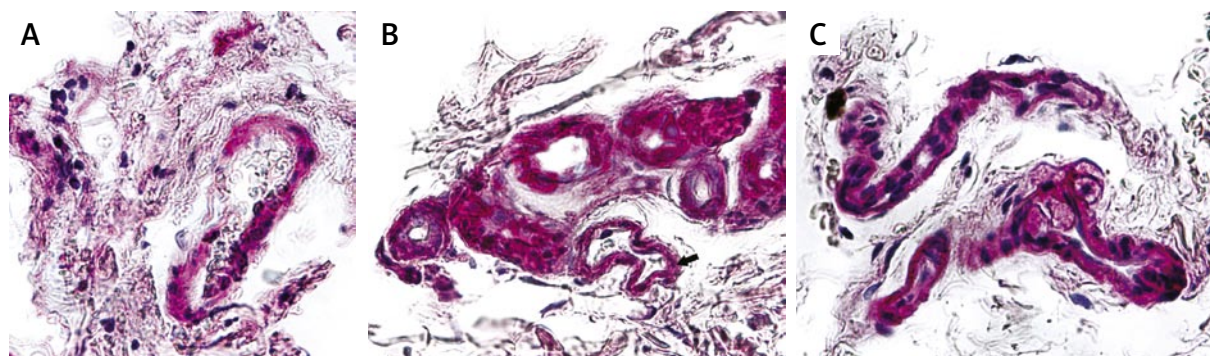


Fig. 3. Walls of vessels with focal PAS positive material. (A) Patient 1 (muscle × 400); (B) Patient 2 (arrow) (skin × 400); (C) Patient 3 (muscle × 400)

Similar ultrastructural changes were visible in the majority of muscle fibrils in patients 1 and 2. Myofibrils were almost completely destroyed by accumulation of free glycogen granules, as well as in vacuoles of various size and in lysosomes (Fig. 4). In patient 1, these pathological changes were more intensive than in patients 2 and 3 (Fig. 5). In patient 3, some muscle fibrils showed intralysosomal glycogen and only a few glycogen granules in the sarcoplasm. Autophagic vacuoles were visible occasionally (Fig. 6). In the wall of blood vessels of muscle and skin biopsies the depositions of glycogen were also visible. In the cytoplasm of endothelial cells, pericytes and smooth muscle cells, free glycogen granules as well as those accumulated in lysosomes were frequently observed (Fig. 7A, B).

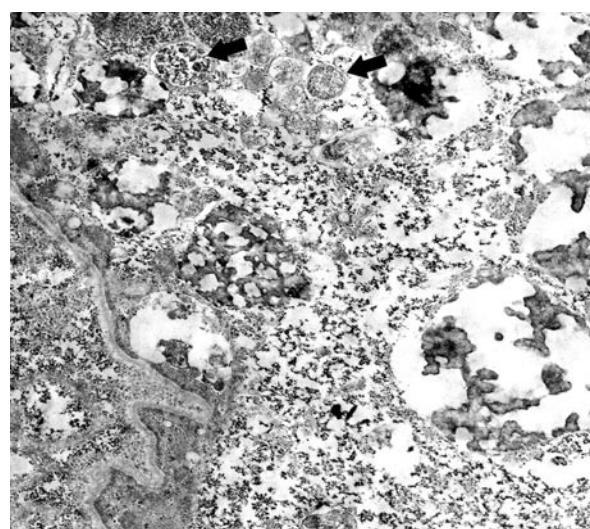


Fig. 4. Patient 1. Massive glycogen deposits in lysosomes (arrows) and those free, without limiting membranes as well as vacuoles in the damaged muscle fibril

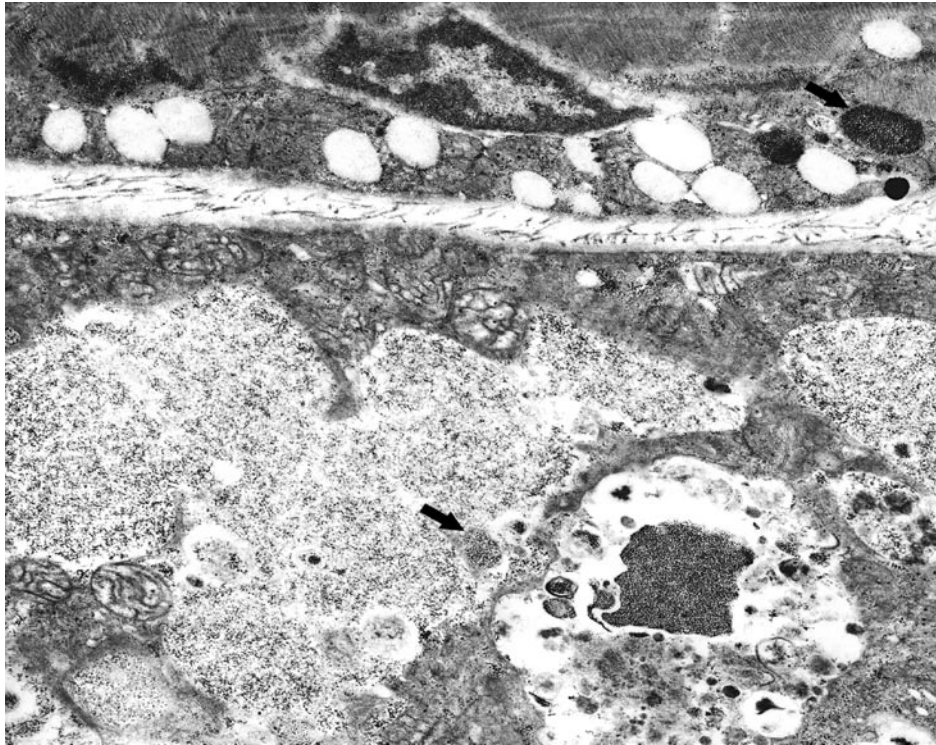


Fig. 5. Patient 2. Muscle fibril with deposits of glycogen in lysosomes (arrows) and sarcoplasm. Various vacuoles also visible

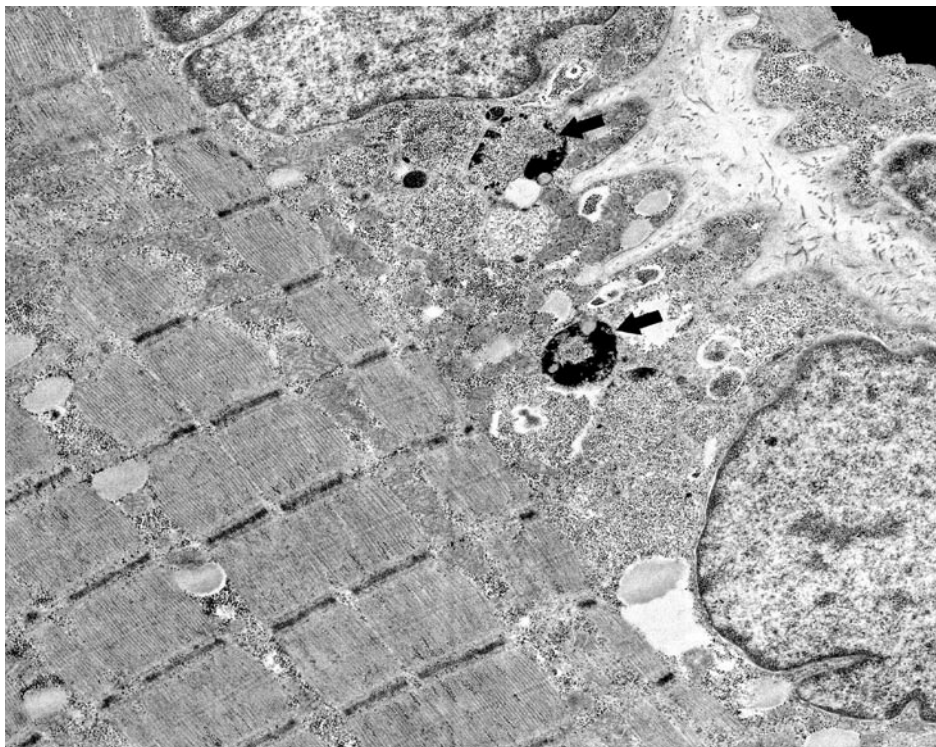


Fig. 6. Patient 3. Intralysosomal (arrows) and free glycogen in sarcoplasm beneath sarcolemma of muscle fibril

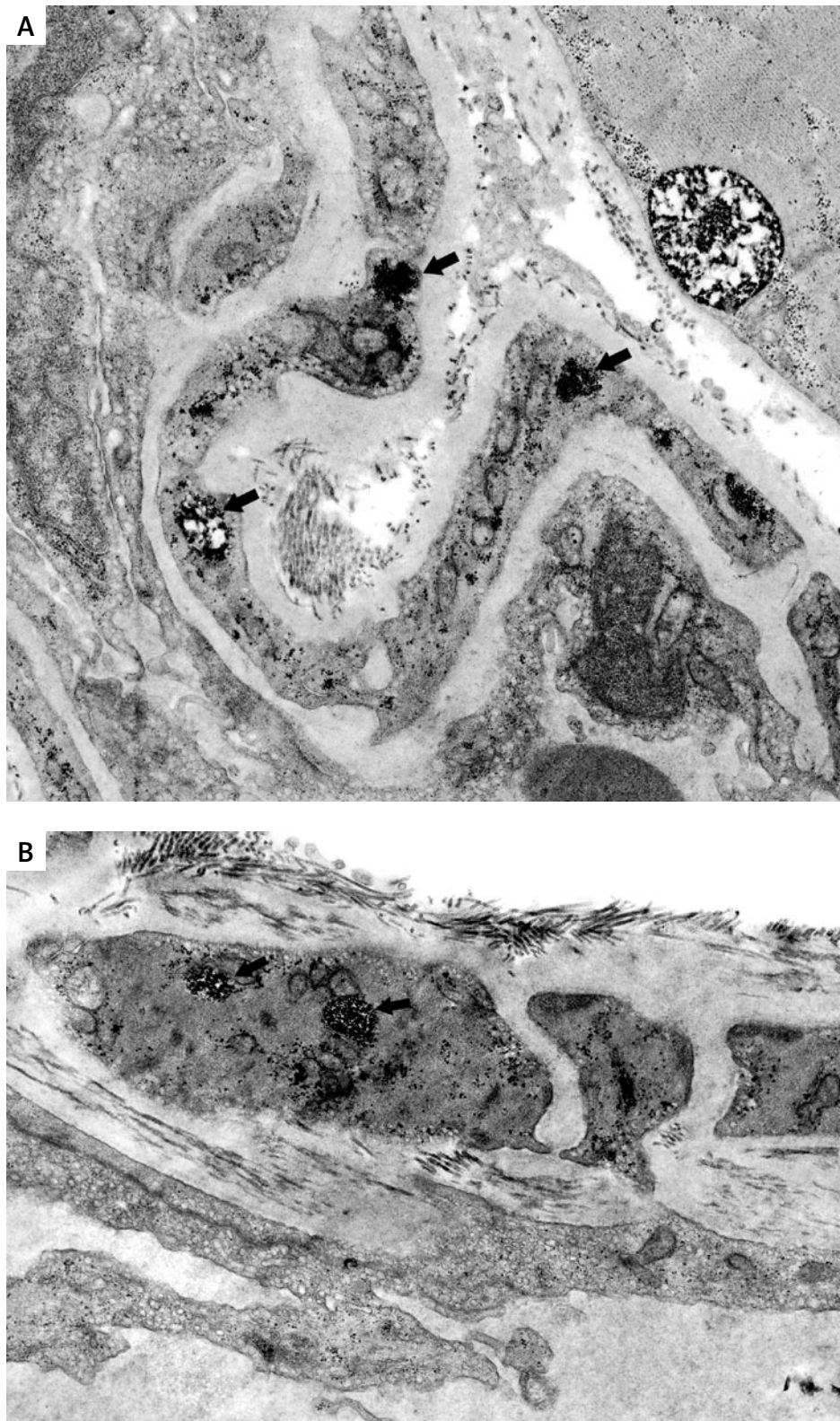


Fig. 7. Patient 2. (A) Blood vessel showing deposits of glycogen in pericyte (arrows); (B) Blood vessel with glycogen in smooth muscle cells (arrows)

Discussion

Clinical manifestations, respiratory failure and muscle weakness were similar in all investigated patients. Similar clinical symptoms have been described by numerous authors in patients with deficiency of acid maltase [15,17]. The character and intensity of clinical manifestations differed between patients. The respiratory failure was more pronounced in patient 1 (aged 19) than in patients 2 (aged 31) or 3 (aged 29). The former (patient 1) showed generalised muscular weakness with more severe involvement of the limbs and respiratory muscles. The course of the disease was acute (patient 1), subacute (patient 2), and mild (patient 3). Clinical pictures did not correlate with the ratio of GAA activity in leukocytes and DBS (Table I) assayed *in vitro* with artificial substrate.

Morphological examination of the muscle and skin biopsies displayed vacuolar degeneration of muscles, abundant glycogen inside of muscles and in the walls of capillaries. Glycogen deposits were observed in lysosomes and as free glycogen deposits or in vacuoles of various size. Similar abnormalities were observed in several types of glycogenosis, but only in Pompe disease and Danon disease was glycogen visible in lysosomes [3,4,11,12,15-17].

The intensity of glycogen storage, vacuolar degeneration and general severity of the disease were reported to be dependent on the degree of acid alpha-1,4-glucosidase deficiency [5,14]. In our observation, neither the intensity of clinical symptoms nor storage of glycogen and vacuolar degeneration were related to the degree of the enzyme deficiency. The lowest ratio of GAA activity in DBS and L (0.05 and 0.07, respectively) was recorded in patient 2 (Table I; Fig. 2), while histopathological and clinical abnormalities were most strongly manifested in patient 1 (0.12 and 0.07, respectively). It is known that there is a spectrum of cellular glucosidases with optimum pH at different values; therefore the measurement of GAA activity at pH 3.8 and in the presence of maltase-glucoamylase (MGA) inhibitor (e.g. acarbose) is of essential importance in the diagnosis of GSD type II. Since we did not assay the activity of all enzymes related to glycogenolysis and glycogenesis, the combined deficiency of GAA and another enzyme could not be ruled out. On the other hand, the function of GAA cannot be limited only to lysosomal degradation of glycogen. Orth and Mundegar [11] suggested that GSDs cause lysosomal proliferation, as well as

affect endosomes, the trans-Golgi network and vesicle population linked to lysosomes. They showed that the intensity of anti-LAMP-1 (lysosomal associated membrane protein-1) staining corresponded with the degree of vacuolation and glycogen storage. A link between the lysosomal membrane function and GAA activity seems to be possible. What is more, GAA plays an important role in the regulation of lysosomal autophagy [1]. An intensive reaction of ubiquitin around vacuoles in muscle biopsies taken from our patients seems to confirm the damage of membrane proteins.

GAA activity does not appear to be the only factor that determines the severity of GSD II.

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