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Remodelling of skeletal muscle cells in children with *SCO2* gene mutation – ultrastructural study

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Abstract

Mitochondrial protein coded by the SCO2 *gene is involved in the process of assembly of mitochondrial cytochrome c oxidase (COX). Progressive cardiomyopathy, neuropathy and lactic acidosis are presented by infants with* SCO2 *gene mutations. Only a dozen patients with this gene mutation have been reported in the literature. Muscle ultrastructure is mentioned only in a few case reports.*

The aim of this study was to search for typical ultrastructural features in 11 skeletal muscle specimens from Polish patients bearing SCO2 *gene mutations. Ultrastructural analysis confirms domination of atrophic and degenerative changes, including atrophic muscle fibres of irregular shape with folding of basal lamina and numerous papillary projections containing altered mitochondria, glycogen granules and degenerated organelles. Advanced disorganization of myofibrils and abnormalities of mitochondria were often found. Myeloid structures, vacuoles, and lipid accumulation were seen only sporadically. Those findings may be attributed to neurogenic atrophy visible in light microscopy.*

Our observations confirm that mutations in the SCO2 *gene are frequently associated with the neurogenic pattern of skeletal muscle involvement accompanied by mitochondrial abnormalities.* SCO2 *gene mutation should be included in differential diagnosis in children with such a pattern; however, lack of neurogenic changes does not exclude* SCO2 *gene mutation.*

Key words: SCO2 *mutations, skeletal muscle, ultrastructure, mitochondrial abnormalities.*

Introduction

Mitochondrial protein coded by the *SCO2* (Synthesis of Cytochrome Oxidase 2; OMIM 604272) gene is involved in assembly of mitochondrial cytochrome c oxidase (COX) and has a role in copper delivery [5,15]. Progressive cardiomyopathy, floppiness and lactic acidosis are presented by infants with *SCO2* gene mutations. Since the first description in 1999 [6] no more than 15 affected patients have been reported in the literature [18]. Results of ultrastructural studies are mentioned only in three case reports [2,19].

In 1997 we observed two infants with spinal muscular atrophy-like (SMA-like) muscle lesions and

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COX deficiency in muscle [7], who appeared later to carry *SCO2* gene mutations. Since then, 12 such cases have been found among patients hospitalized in our hospital in the period of 1994-2008, by retrospective [4,8,9,17] and prospective study [unpublished data].

The aim of this work is a systematic search for typical ultrastructural features in 12 skeletal muscle specimens from patients bearing *SCO2* gene mutations.

Material and Methods

All available muscle specimens of patients carrying pathogenic mutations in both alleles of the *SCO2* gene were included in the study. Muscle biopsy was performed at the age of 1.5 to 15 months. Clinical course of the disease strongly suggested mitochondrial encephalomyopathy in all patients.

An example of typical clinical course in our patients is shown below. The molecular background of the disease was recognized in this case 8 years after death [4,8,9,17].

Case report (Patient 1)

She was the second child of non-consanguineous healthy parents. Her older brother is healthy. The pregnancy, delivery and development during the first 4 months of life were uneventful. Later, the mother recalled that there were some difficulties with feeding from the second month of age. The disease started with mild floppiness and psychomotor retardation at the age of 4-5 months and was slowly progressive. There was no relationship to any stressful situations such as infection or vaccination. At the same time, laryngeal stridor developed. At the age of 8 months the girl was not able to keep her head up, sit or turn over. Her lower legs were hypotonic without spontaneous movements but her upper legs were transiently hypertonic. She did not keep toys and did not follow things with her eyes. Episodes of tonic fits and dissociation of eye movements were observed. Stridor became very remarkable, leading to respiratory insufficiency during anxiety. Long epiglottis was suspected in directoscopy. Electrocardiogram and echocardiogram were normal. No changes were found in the fundus. CT showed some features of mild brain atrophy. Mild EEG pathological changes were assessed as in the normal range. Selective screening for metabolic disorders revealed only a metabolic acidosis (pH 7.13, pCO_2 31.2 mmHg, HCO_3 – 15.9 mmol/L), low cholesterol level (75 mg%) and hyperlacticaemia (2.2 – 5.5 mmol/L, control 0.4-0.8 mmol/L) with hyperalaninaemia (521 µmol/L, control value below 380 µmol/L).

At the age of 10 months the girl was hospitalized for further metabolic investigation. All biochemical results were inconclusive. Only a small increase of oxoglutaric acid was found by GC-MS analysis of urinary organic acids profile. Lysosomal enzyme activities, serum biotinidase activity, and labelled palmitate oxidation were normal. A mild decrease in serum free carnitine concentration was found (free 19 µmol/L, total 37 µmol/L). Plasma lactate levels were not remarkably increased during fasting as well as intravenous glucose loading (1.4 mmol/L and 2.7 mmol/L respectively, normal value below 2.0 mmol/L). During a month of hospitalization the clinical condition worsened. There was severe muscle hypotony with episodes of increased muscle tonus, especially proximal upper legs. Seizures appeared three times. Protracted fever resistant to treatment was seen although no infection could be confirmed. Tracheostomy had to be performed due to chronic respiratory insufficiency.

Muscle biopsy showed features characteristic of spinal muscular atrophy (SMA) (Fig. 1). Respiratory chain function was not assessed.

The girl was sent to her local hospital for continuation of artificial ventilation. At discharge the diagnosis was spinal muscular atrophy (although there were discrepancies between the morphological

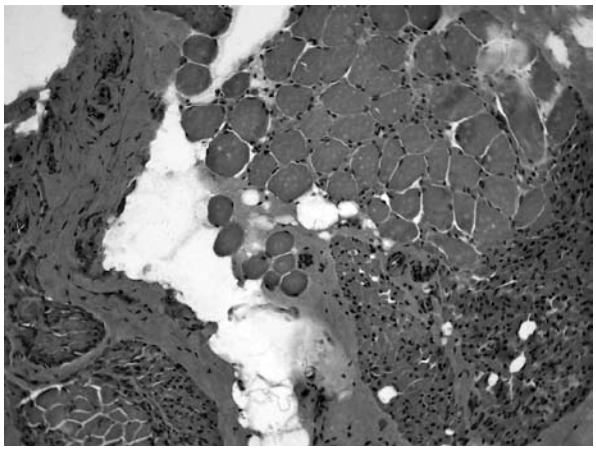

Fig. 1. Muscle biopsy in a patient carrying pathogenic mutations on both alleles of *SCO2* gene. H&E

picture of the muscle and the clinical course of the disease). Further molecular investigations did not reveal deletion in *SMN* and *NAIP* genes excluding SMA. Outcome is unknown.

Six years later a systematic search for mutations in the *SCO2* gene showed presence of homozygous missense c.C1280T (p.E140K) [4].

Ultrastructural study of the muscle samples

For ultrastructural study, the tissue samples from skeletal muscle biopsies were fixed in 2.5% cold glutaraldehyde, washed in cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated in graded alcohols and embedded in Epon 812. Semithin sections were stained with toluidine blue. Transverse and longitudinal ultrathin sections were counterstained with uranyl acetate and lead citrate and examined in a JEOL 1500 electron microscope (Tokyo, Japan).

Results

The most prominent ultrastructural changes were related to muscle fibre atrophy accompanied by mitochondrial degeneration (summarized in Table I).

Highly atrophic muscle fibres of abnormal size and shape often displayed large, centrally located nuclei and disorganized myofibrillar component (Fig. 2). On longitudinal section, a group of a few nuclei occurred at the periphery of the sarcoplasm along the outer membrane (Fig. 3). The nuclei often exhibited infoldings of nuclear membrane and condensed peripheral chromatin. Occasionally, the nucleus was entirely located within advanced folding of the cytoplasm (Fig. 4).

Atrophic muscle fibres exhibited numerous papillary projections on their surface membrane (Fig. 5). These surface projections usually contained altered mitochondria, vacuoles and degenerated organelles (Fig. 6). Sometimes, the papillary projections and adjacent subsarcolemmal space revealed accumulation of abnormal, dark, irregular mitochondrial profiles and small vacuoles (Fig. 7).

Most mitochondria exhibited structural abnormalities concerning their size, shape and internal matrix. The altered mitochondria were irregularly disposed between myofibrils (Fig. 8A) or aggregated into small clusters within the central part of the sarcoplasm. Many of them were elongated (Fig. 8B) or displayed an irregular, bizarre configuration (Fig. 8C). Occasionally, the mitochondria exhibited completely destroyed matrix and cristae, with lamellar structure (Fig. 8D). The abnormal mitochondrial profiles of enlarged shape and increased matrix density with disorganized cristae were often gathered along the outer border of the fibres (Fig. 9A,B). Subsarcolemmal space often contained abnormal mitochondria, vacuoles, myeloid structure and/or endocytic vesicles (Fig. 10).

Numerous muscle fibres demonstrated focal or diffuse disorganization of myofibrils accompanied by irregularly disposed altered mitochondrial profiles (Fig. 11). Highly atrophic fibres with total disorganization of myofibrils and numerous papillary projections of various size were observed (Fig. 12). Some regions of fibres were completely deprived of myofibrils and contained only a few scattered mitochondria, vacuoles and glyco-

Table I. Summarized data of skeletal muscle ultrastructure of patients with *SCO2* gene mutations

Number of patient, gender (F/M)	Age at biopsy	Clusters of nuclei	Papillary projections	Loss of myofibrils	Altered MT	Other abnormalities
1. F	10 months	+	++	++	+++	Myeloid structures
2. F	9 months	+		+	+	Lipid droplets
3. M	9 months		+	+		Myeloid structures
4. M	6 months		+++	++	+++	
5. M	12 months		+++	+++	+++	Myeloid structures
6. F	4 months		+	++	++	Lipid droplets, cytoplasmic body
7. F	10 months		++	++	+++	Lipid droplets, myeloid structures
8. F	7 months		++	++	+++	Myeloid structures
9. F	14 months	+	++	++	++	
10. F	9 ½ months		+	++	+	
11. M	5 weeks		+++	++	+++	

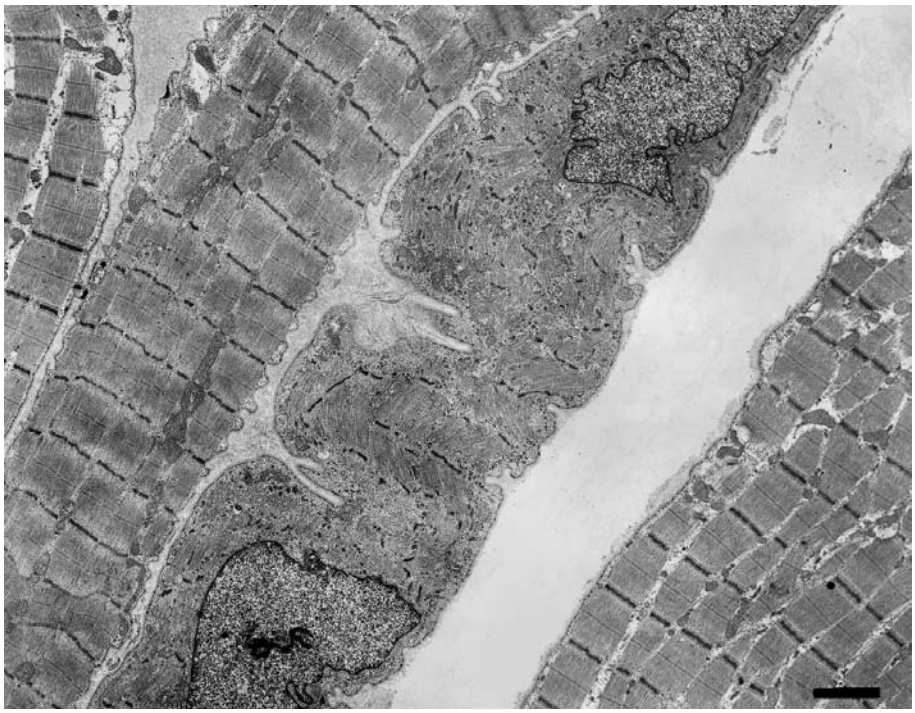

Fig. 2. Atrophic muscle fibre of irregular shape with two large nuclei. Bar: 2 µm

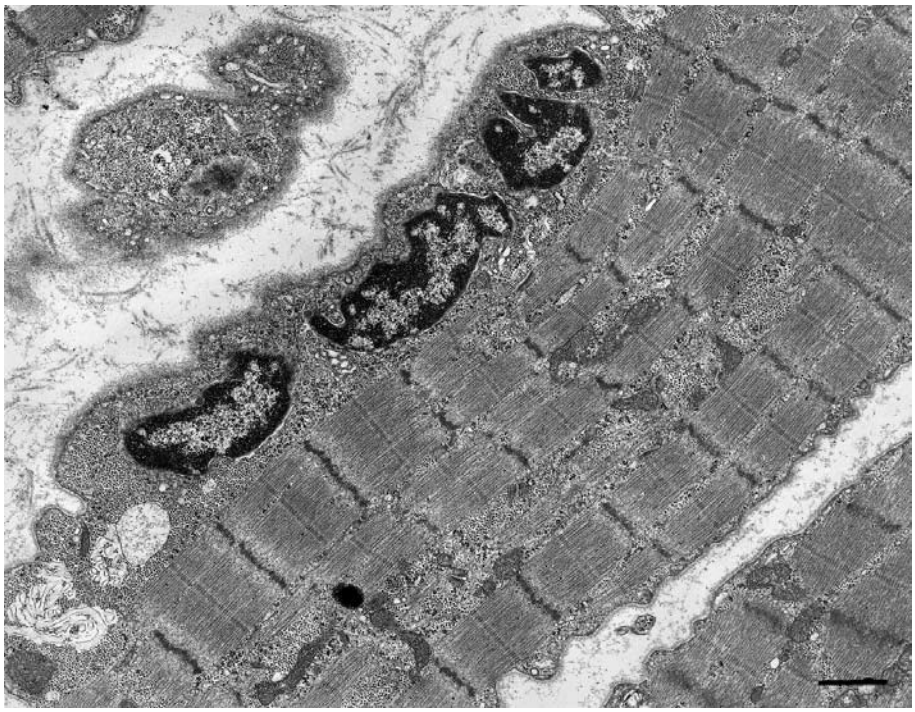

Fig. 3. Muscle fibre exhibiting collection of several nuclei with condensed peripheral chromatin. Bar: 1 µm

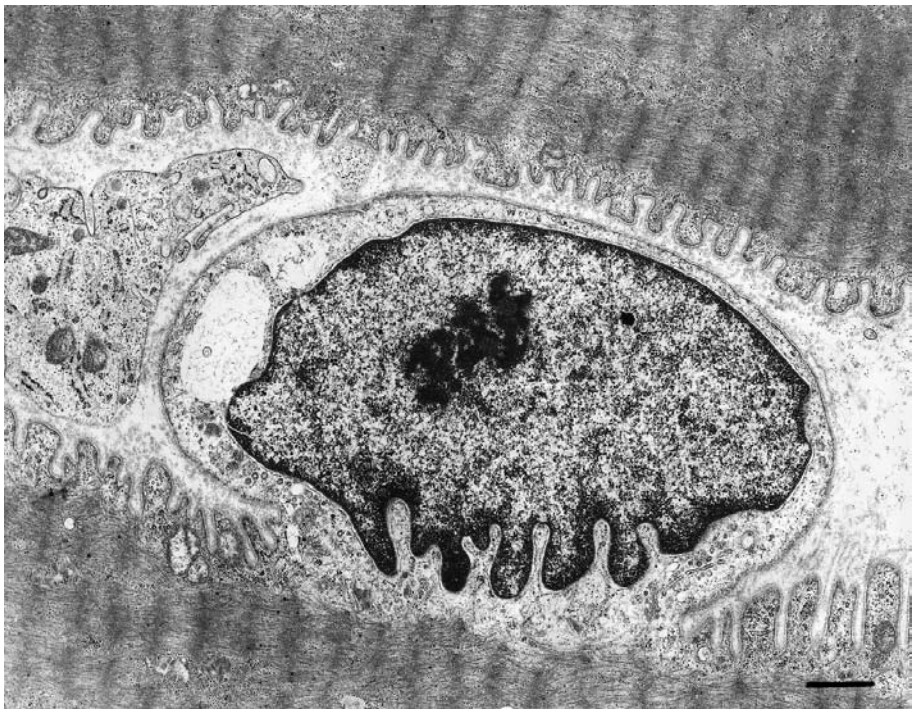

Fig. 4. Nucleus located within folding of cytoplasm of atrophic muscle fibre. Bar: 2 µm

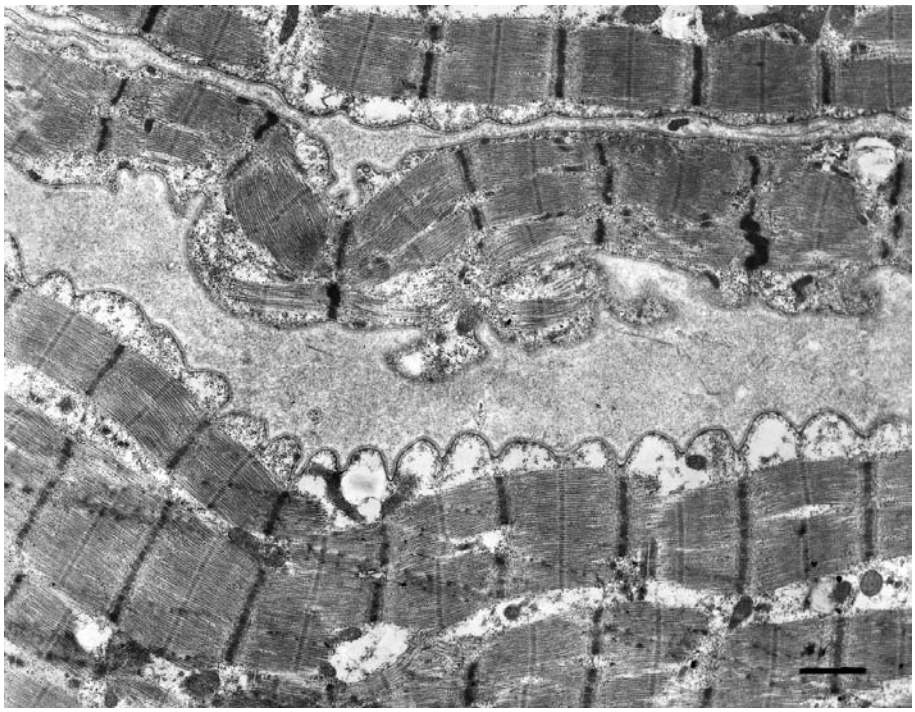

Fig. 5. Atrophic fibres of reduced size with numerous surface papillary projections. Bar: 1 µm

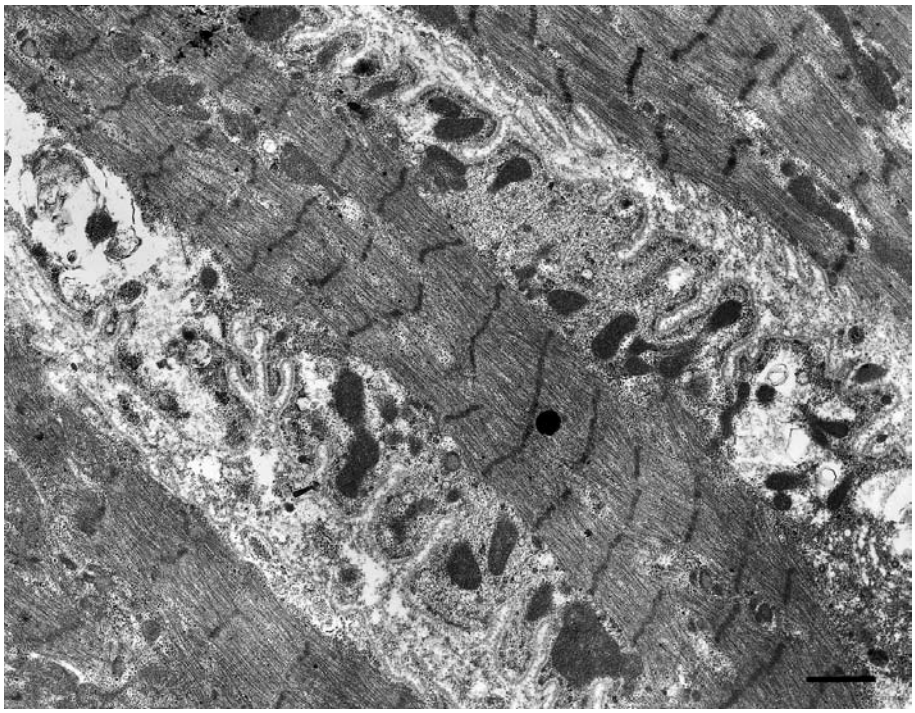

Fig. 6. Highly atrophic muscle fibre with numerous papillary projections containing dark mitochondria of various size. Bar: 1 µm

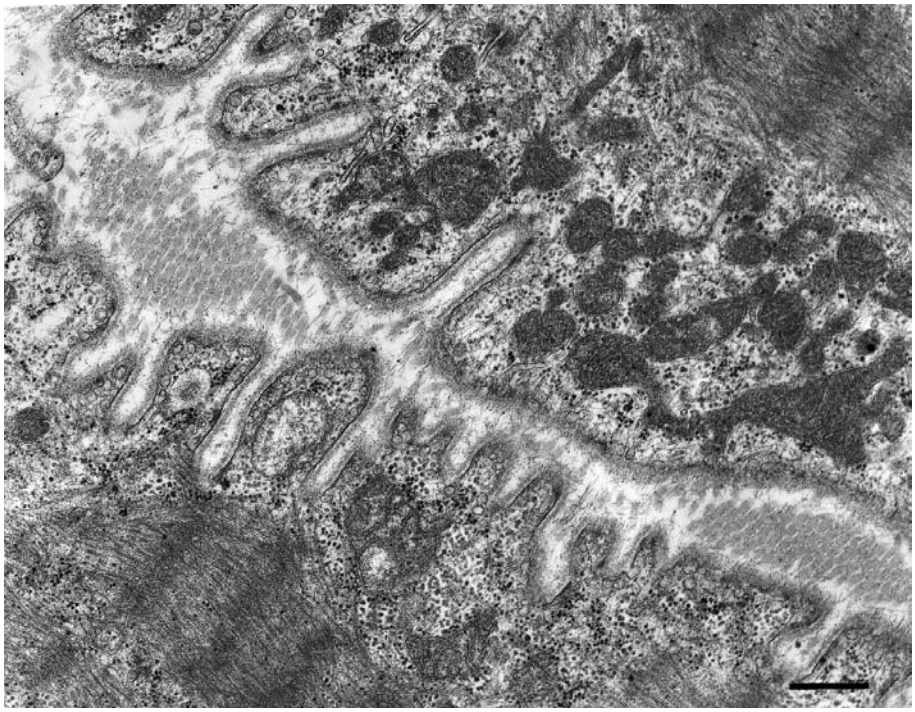

Fig. 7. Accumulation of mitochondrial profiles of various size and shape in papillary projections and subsarcolemmal space. Bar: 500 nm

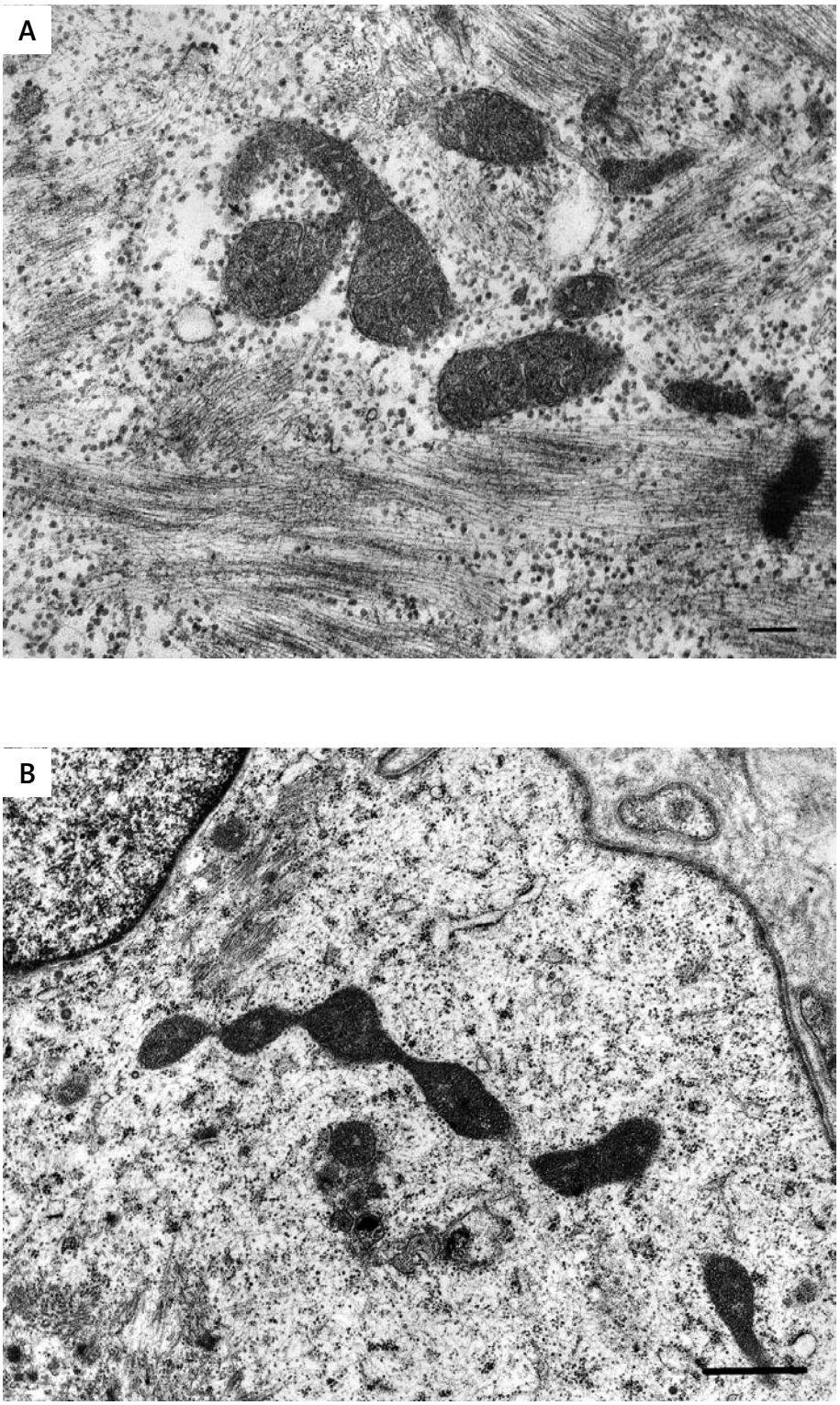

Fig. 8A-B. Alteration of mitochondria. A. Mitochondria of various shape in the central part of sarcoplasm. Bar: 200 nm. B. Elongated, polygonal mitochondrium. Bar: 1 µm

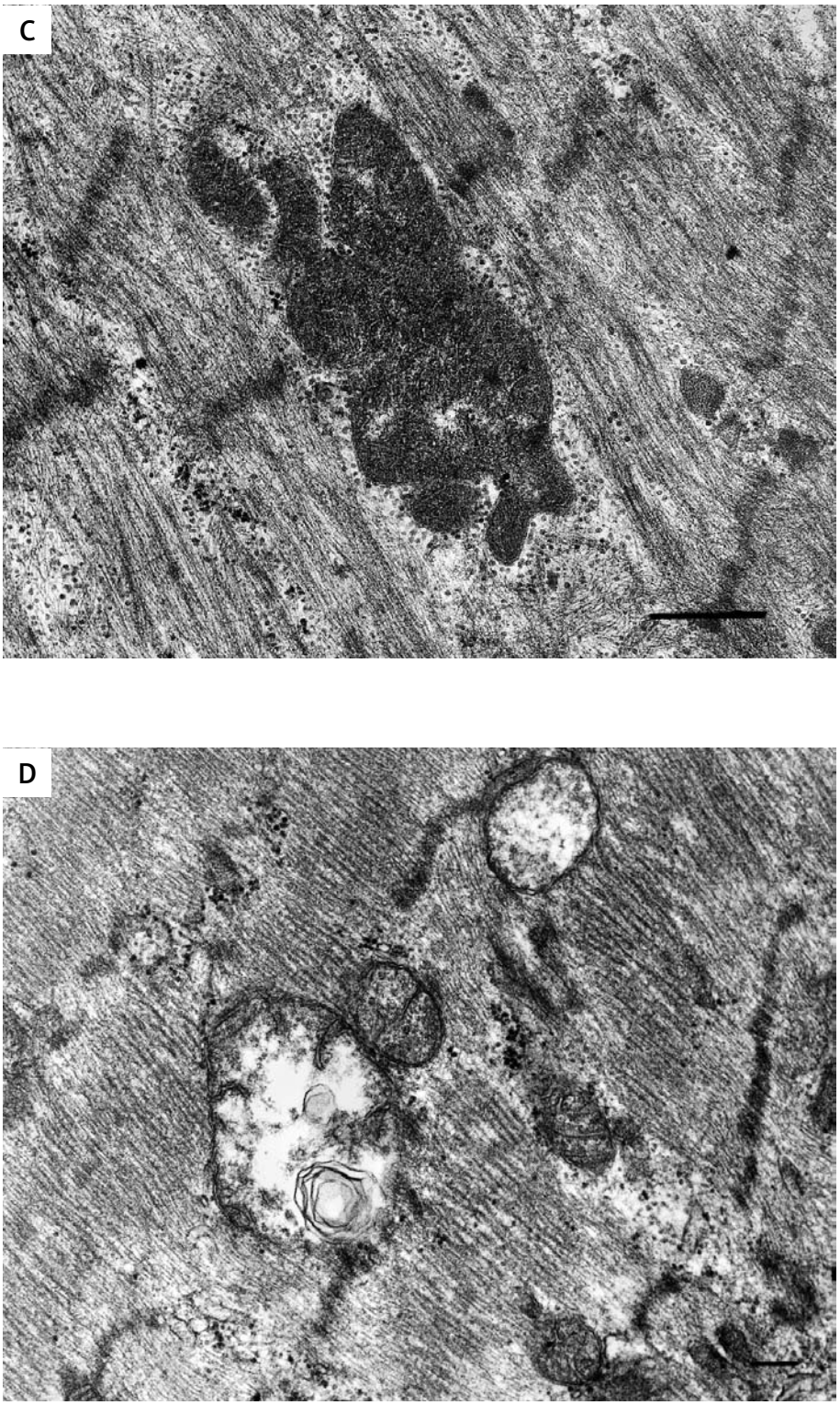

Fig. 8C-D. Alteration of mitochondria. C. Enlarged mitochondrium of bizarre shape and condensed matrix. Bar: 500 nm. D. Enlarged mitochondrium with destroyed cristae and lamellar structures. Bar: 200 nm

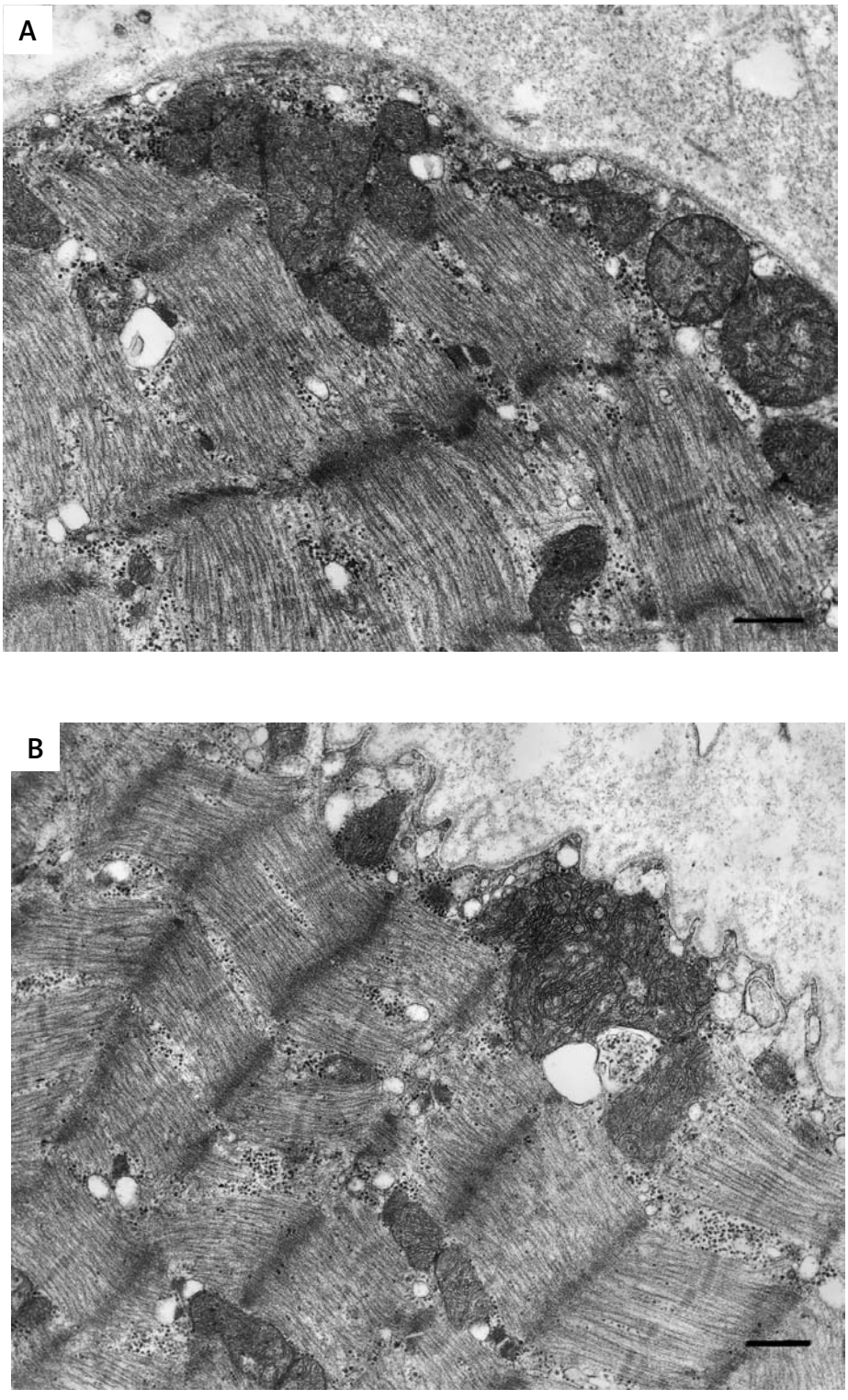

Fig. 9. Accumulation of altered mitochondria in subsarcolemmal space. A. Densely packed, enlarged, dark mitochondrial profiles with irregular cristae along outer membrane. Bar: 500 nm. B. Enlarged mitochondrial profile exhibiting changes of their internal structure with aberrant configuration of cristae. Bar: 500 nm

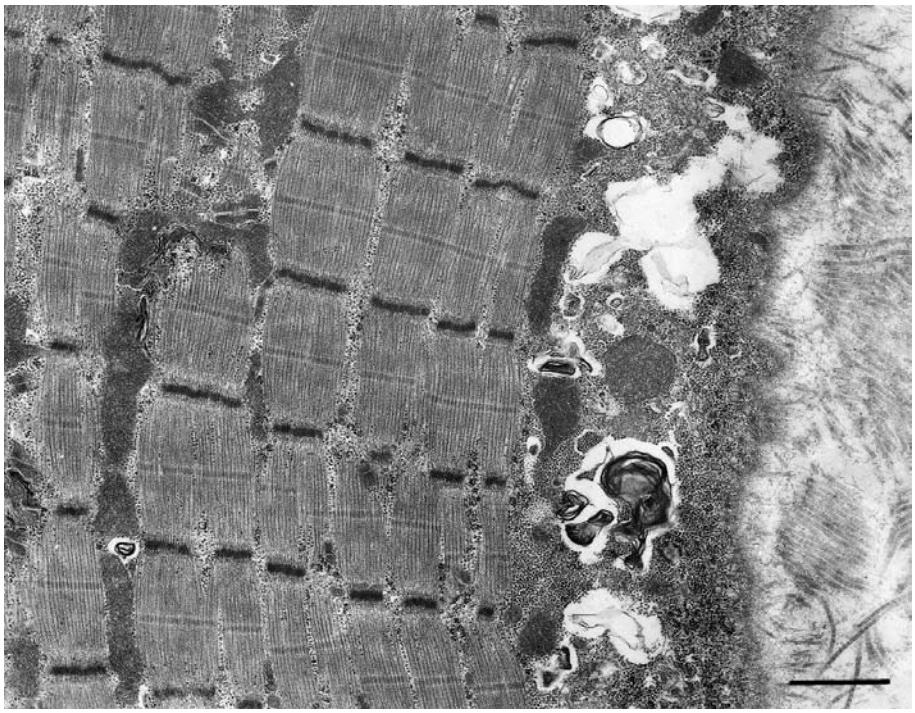

Fig. 10. Dark mitochondria, myeloid structures and vacuoles in subsarcolemmal space. Bar: 1 µm

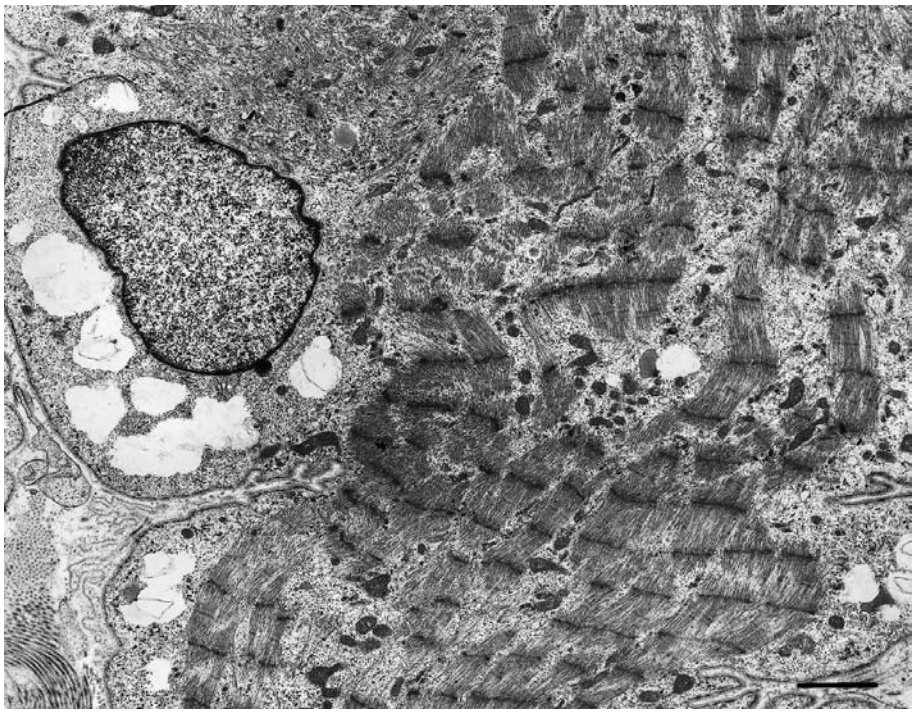

Fig. 11. Atrophic muscle fibres with irregularly oriented and disorganized myofibrils and irregularly disposed small mitochondria. Bar: 2 µm

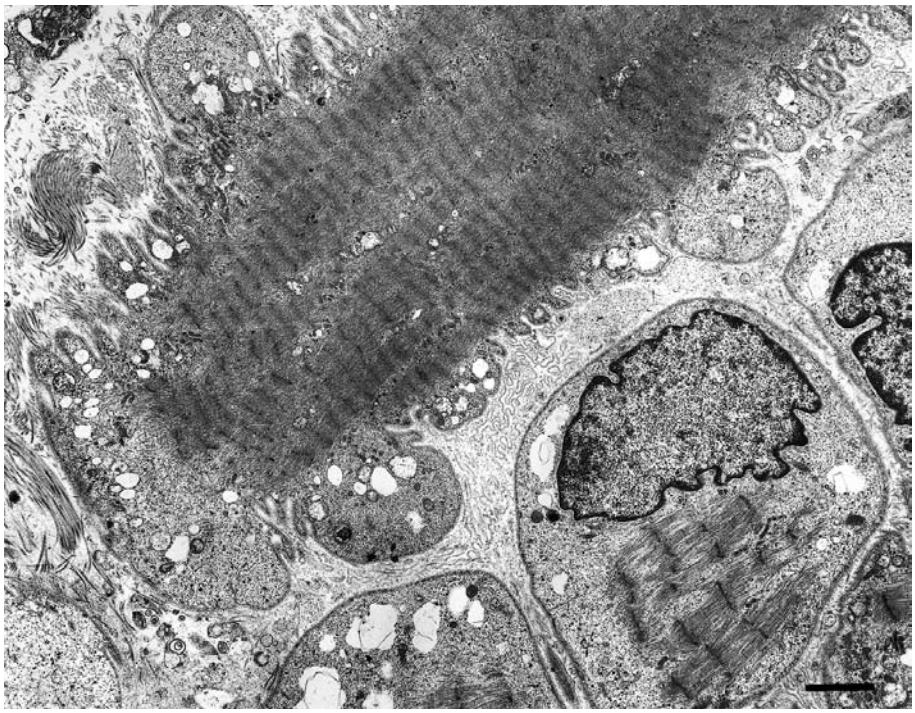

Fig. 12. Highly atrophic muscle fibre with total disorganization of myofibrils and numerous papillary projections of various size. Small fragments of muscle fibres in neighbourhood. Bar: 2 µm

gen granules (Fig. 13). Individual atrophic muscle fibres were surrounded by redundant loops of basal lamina.

In five cases, the muscle fibres demonstrated an excessive amount of lipid droplets (Fig. 14), lipofuscin granules and membranous structures. Other structural abnormalities such as cytoplasmic body, nemaline rods or tubular aggregates were encountered only occasionally. Apoptotic changes manifested by nuclear condensation, fragmentation and formation of membrane bound apoptotic bodies were rarely encountered.

Discussion

Prevalence of SCO_2 deficit in the Polish population is not known and the detection level is at present inadequate [1]. Due to rapid and unfavourable course of the disease, and lack of specific biochemical markers, many children die without an established diagnosis, so parents remain at risk of bearing affected children in accordance with autosomal recessive inheritance. In our experience, several (but not all) cases of *SCO2* gene mutations were eventually detected in children presenting SMA-like lesions in skeletal muscle biopsy and/or autopsy [10,11]. The phenomenon seems to be limited to homozygous E140K *SCO2* mutation, and was not seen in several other mitochondrial disorders, including *SURF1* gene mutations [12]. The same finding has been reported by other authors [3,13,14,16]. This observation indicates that in selected patients, light microscopic assessment may be helpful in directing the molecular diagnostic search towards the *SCO2* gene.

Ultrastructural analysis confirms predominant atrophic and degenerative changes of muscle fibres, which may be attributed to neurogenic atrophy observed in light microscopy. Highly atrophic muscle fibres presented a disorganized myofibrillar component accompanied by mitochondrial abnormalities. Mitochondrial involvement seems non-specific and their number, shape, and structure do not provide additional clear-cut diagnostic evidence.

The results of our study are in agreement with other sporadic data found in the literature. Jaksch et al. [2] reported morphological and ultrastructural examination of one *SCO2*-deficient infant at autopsy. They found proliferation of abnormal mitochondria with vacuolated cytoplasm in the myocardium and skeletal muscle, and no RRFs. Vesela et al. [19] did not find any ultrastruc-

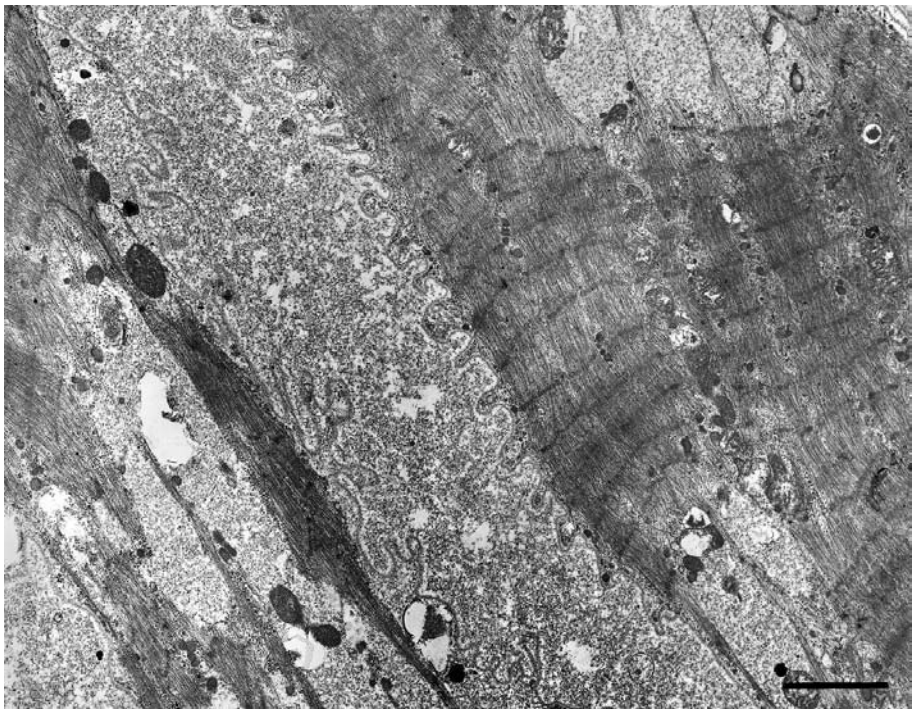

Fig. 13. Focal loss of myofibrils. Wide, empty intramyofibrillar spaces containing only scattered mitochondria. Bar: 2 µm

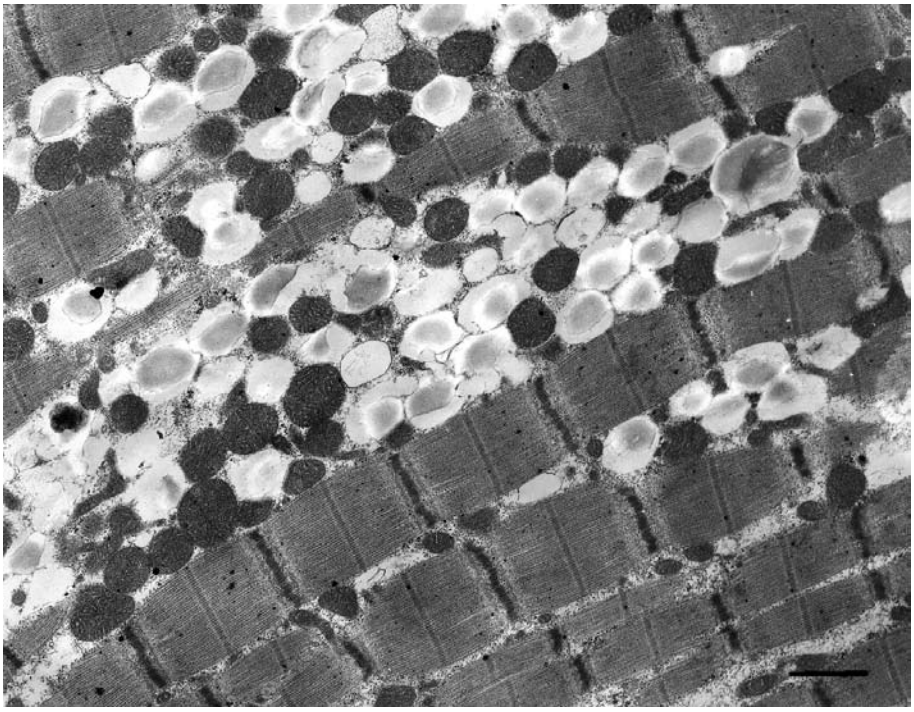

Fig. 14. Extensive accumulation of lipid deposits. Bar: 1 µm

tural anomalies in two examined muscle specimens of patients with *SCO2* gene mutations. Axonal and myelin destruction in skin dermal nerve (one patient) and in the sural nerve, with remyelinization and axonal sprouting (another patient) was found. Immunostaining of cardiocytes (2 samples) showed enlargement and increase in number of mitochondria. On ultrastructural study mitochondria were frequently densely aggregated with close contact with their external membrane. No ultrastructural anomalies were found in the liver and kidney (one sample, each). In our study, mutation in *SCO2* was associated with progressive hypertrophic cardiomyopathy only in one patient.

Our observations confirm that mutations in the *SCO2* gene are frequently associated with neurogenic pattern of skeletal muscle involvement. *SCO2* gene mutation should be included in differential diagnosis in children with such a pattern; however, lack of neurogenic changes does not exclude *SCO2* gene mutation.

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