

Lipid raft disease? A new severe congenital myopathy

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Abstract

We report a 5-year-old girl with a unique neuromuscular disorder manifested by early onset of the disease, delayed motor development, joint contractures, dysmorphism, cobbler's chest, generalized muscle hypoplasia and weakness. Morphological examination revealed muscle cell immaturity and the appearance of multilamellar myelin-like structures within and outside the sarcolemma. Overexpression of aberrant lipids on the surface of affected muscle cells may suggest some failure in lipid raft formation.

Key words: congenital myopathy, lamellar bodies, lipid raft remodelling

Introduction

Ultrastructural abnormalities of the plasma membrane in muscle cells have received little attention compared to the pathological changes in other muscle cell components. However, it is now widely accepted that abnormal plasma membrane lipid raft structure provides important clues to the diagnosis of some neuromuscular disorders [9]. For this reason we present a form of congenital myopathy that does not resemble any previously diagnosed ones. In a child affected since birth, muscle biopsy showed structural abnormalities that implied a defect in the lipid raft of the sarcolemma.

Case report

A girl T.G. aged 5 years was born as the fourth child to her mother with delivery complicated by placental ablation. Birth weight was 2800 g and the Apgar score was 9/5 points. She was floppy at birth and her motor development was delayed. She did not sit unsupported until 20 months and she started to walk at 3 years. The girl was repeatedly hospitalized in an intensive care unit of a paediatric department due to recurrent and prolonged broncho-pulmonary infections. At the age of 5 years she was admitted for evaluation to the Neurological Unit in the Paediatric Department in Katowice.

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When she was first examined at the age of 5 years her body weight was 13 kg, i.e. below the third percentile. Height was also below the third percentile. Her musculature was generally thin and undeveloped. She had a long face, slight bilateral ptosis, and arched palate and chest deformity. There was generalised muscle weakness with flexion contractures of hips, knees and ankles. Her tendon reflexes were reduced. She did not walk.

Her non-consanguineous parents and older siblings were asymptomatic.

Laboratory findings were entirely within normal limits except for elevated ESR (erythrocyte sedimentation rate). Electrocardiogram (ECG) revealed features of right ventricular hypertrophy. Electromyography (EMG) examination showed a myopathic pattern. Electroencephalography (EEG) was normal. Her mental development was slightly delayed (IQ 85). The girl rapidly deteriorated due to left inferior atelectasis and was admitted to the Department of Thoracic Surgery. She died a year later apparently following a mild respiratory infection and cardiorespiratory insufficiency. The parents did not consent to autopsy.

Material and Methods

Muscle biopsy taken from *vastus lateralis* was analysed by light and electron microscope. Serial frozen sections for light microscopy were stained according to standard techniques. For immunohistochemistry, 8 µm cryostat sections were stained by direct immunofluorescence methods using monoclonal anti-desmin (Daco), anti-dystrophin, anti-fibronectin, anti-laminin and anti-collagen IV (Novocastra) antibodies according to previously described methods [1].

For electron microscopy, muscle specimens were fixed in 3% glutaraldehyde in phosphate buffer and postfixated in 1% osmium tetroxide in the same buffer. They were then dehydrated and embedded in Epon. Thin sections double stained with uranyl acetate and lead citrate were examined with a JEM II electron microscope.

Results

Quadriceps muscle biopsy samples showed that numerous small round muscle fibres of 8-10 µm in diameter provided 75% of all muscle fibres and these were intermingled with muscle fibres of a normal diameter. There was no necrosis or degeneration of

muscle fibres and no inflammation. Many muscle fibres showed unstained peripheral border by haematoxylin-eosin (HE) and trichrome staining (Fig. 1). Small muscle fibres occasionally revealed internal nuclei and lack of fibre type differentiation (Fig. 2) but their uniform intense desmin staining (Fig. 3) suggested immaturity.

Immunostaining of muscle fibres demonstrated broad outlines of laminin (Fig. 4), fibronectin (Fig. 5a) and collagen IV (Fig. 5b). Both types of muscle fibres were dystrophin positive (Fig. 5c). The most intriguing finding in epon sections (Fig. 6) was the presence of numerous multilamellar bodies decorating the periphery of small muscle fibres which were not observed in muscle fibres of normal diameter. At the ul-

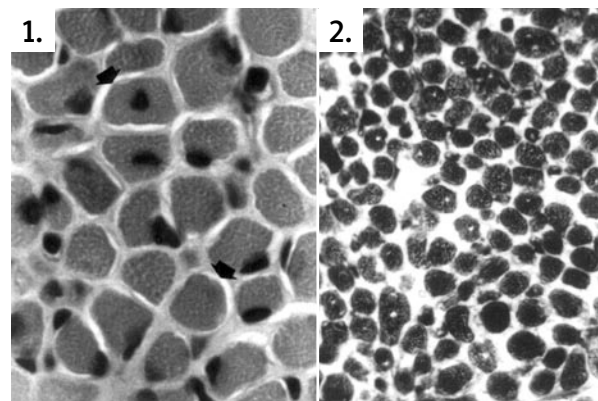


Fig. 1. Muscle fibres indicate unstained peripheral border (arrowheads). HE × 1050

Fig. 2. Small muscle fibres lacking fibre type differentiation. NADH × 448

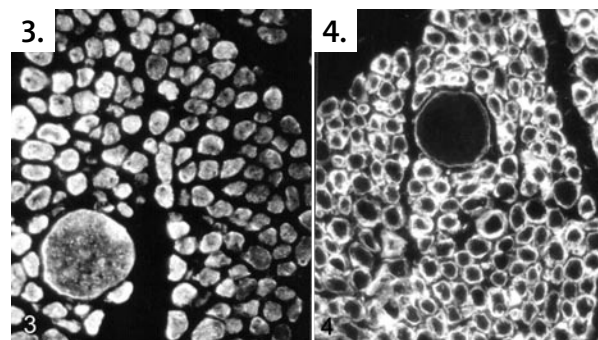


Fig. 3. Intensely desmin staining muscle fibres. × 448

Fig. 4. Immunostaining demonstrates wide outlines of laminin. × 448

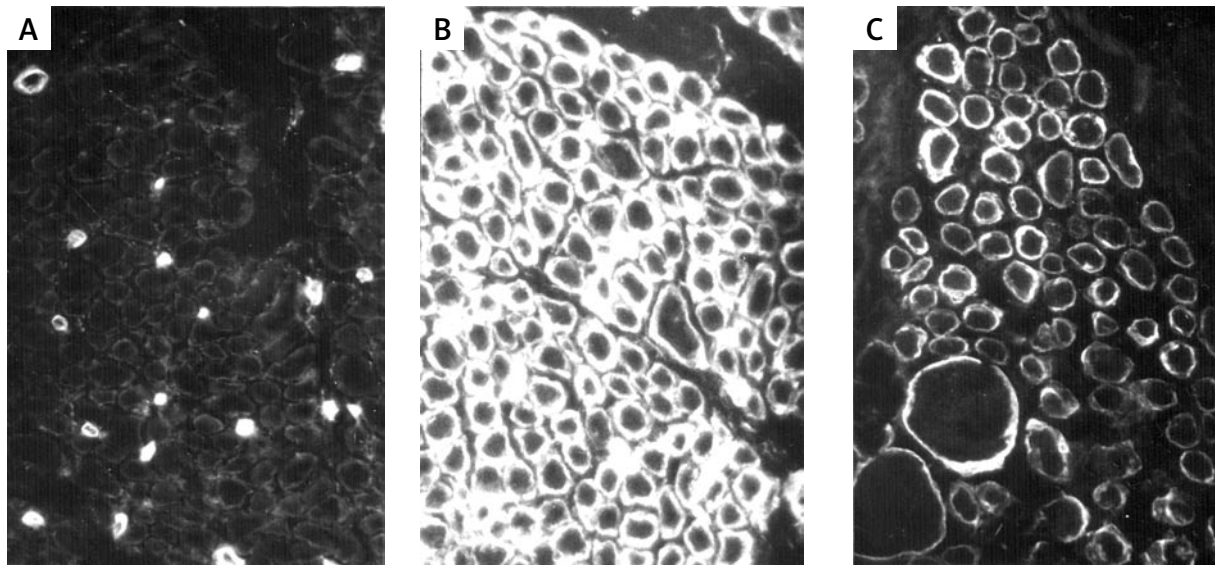


Fig. 5. Irregular widened outlines of fibronectin (A), collagen IV (B) and dystrophin (C). $\times 448$

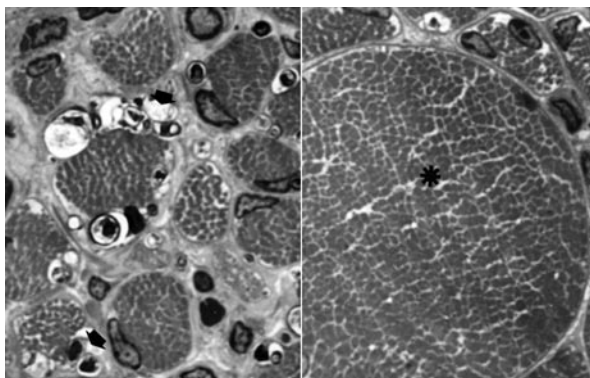


Fig. 6. Lamellar bodies at the periphery of small muscle fibres (arrowhead). Muscle fibre with normal diameter (asterisk) shows smooth surface. $\times 1050$

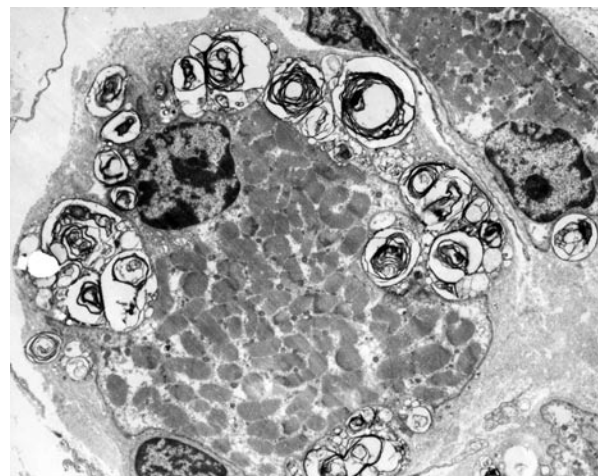


Fig. 7. MLMBs decorate the surface of muscle fibres. $\times 5000$

trastructural level, the most conspicuous finding was the presence of numerous round, rectangular or irregular multilamellar vesicular myelin bodies (MLMBs) of 0.1 to 4 μm in diameter which covered the surface of small muscle fibres (Fig. 7). At higher magnification, the stored material was presented in the form of osmiophilic altered membranous structures, generally lamellar, but also appearing as vacuolated or whorled structures (Fig. 8). Their location was very interesting. Some MLMBs were settled deep inside of large caveolae-like pockets and were closely con-

nected to the plasma membrane. It seems that the external layer of MLMB forms a common membrane with the plasma membrane (Fig. 8).

Other MLMBs were located more superficially and separated from the plasma membrane by basement membrane (Fig. 8). The pockets containing myelin structures were sometimes so deep that they appeared to dissect the muscle cell. Over-expression of caveolae was observed at sites of MLMB contact with the plasma membrane. Except for minor abnormalities the internal architecture of small muscle fi-

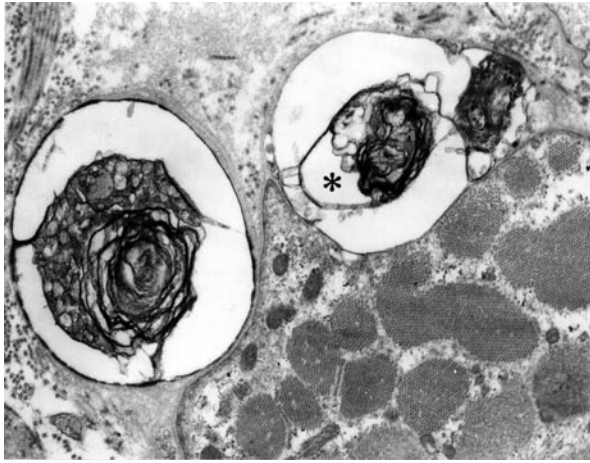


Fig. 8. MLMB (asterisk) is settled inside caveolae-like pocket and is closely connected with plasmalemma. $\times 20\ 000$

bres was unremarkable. They were characterised by single nuclei, close adherence of the sarcolemma to the basement membrane, and normal structure of myofibrils. In a very few muscle cells, intracytoplasmic MLMBs were observed.

Sometimes they were located peripherally near the plasma membrane (Fig. 9). The remaining structures such as fibroblasts, endothelial cells and intramuscular nerve fibres were normal.

Discussion

In the present study we describe clinical, morphological and immunohistochemical features of a new myopathy. Hypotonia at birth, undeveloped muscle mass associated with skeletal dysmorphism, progressive muscle weakness, joint contractures and myopathic pattern of EMG are typical for a congenital myopathy. In addition, chronic respiratory insufficiency is related to skeletal muscle dysfunction. In contrast to these unspecific clinical findings, abnormalities seen in the muscle biopsy are unusual and to our knowledge have never been presented in the literature. Seventy-five percent of muscle fibres showed features of immaturity. It was manifested by small muscle fibre size, single nuclei, lack of enzymatic differentiation and intensive activity of desmin. The most intriguing finding was the presence of lipid raft abnormalities in small muscle fibres. Massive accumulation of multilaminar myelin-like bodies (MLMBs) covered the surface of immature

muscle fibres. They were not visible on the surface of fibres of normal diameter. Multilaminar deposits were abundant and their architecture closely resembled unesterified cholesterol storage in patients with type C Niemann-Pick disease [4]. The plasma membrane (PM) of muscle cells (sarcolemma) consists of a well ordered array of protein and lipids which are mainly composed of cholesterol and sphingolipids [6]. Cholesterol is a major component of PM in mammalian cells. These cells obtain cholesterol in two ways: by taking it up from the environment or *de novo* synthesis. Sphingolipids and cholesterol are synthesized in endoplasmic reticulum and Golgi apparatus and subsequently transported to various membrane components, mostly to PM. Cholesterol and sphingolipids form membrane microdomains are termed rafts [8]. The lipid rafts play an important role in many cellular functions such as adhesion, signalling, motility and membrane trafficking. Caveolae are a specialized form of lipid rafts appearing as 50-100 nm invaginations in the PM of most cell types [2]. The molecular composition of caveolae is different from other membrane domains. The membrane structure is rich in cholesterol, sphingolipids, gangliosides, anchored membrane proteins, and the integral membrane protein caveolin [2,3]. Caveolae are involved in many processes, including cholesterol trafficking within the cell, endocytosis of certain molecules and regulation of signal transduction pathways. To date, three major forms of caveolin have been identified. Caveolin 1 (Cav 1) and caveolin 2 (Cav 2) are expressed in many cell types. Caveolin 3



Fig. 9. Intracytoplasmic location of MLMB. $\times 20\ 000$

(Cav 3) is specific for muscle [10]. Caveolins participate in vesicular trafficking to organize, concentrate and regulate specific lipids [2]. A dramatic reduction of caveolae and expression of caveolin 3 results in limb-girdle muscular dystrophy [5]. Caveolin 2 (Cav 2) is dependent on Cav 1 and in the absence of Cav 1 it undergoes proteolytic degeneration. Therefore, Cav 1 mutant mice are severely deficient in Cav 2, resulting in dramatic lung dysfunction [7]. All these data indicate that PM lipid raft and its specialized forms, caveolae, play an important role in muscle and lung function and both were affected in our patient. Based on the structural alterations that were seen in affected muscle fibres, we speculate that MLMBs, largely coating the surface of myotubes, maintain the fusing process during differentiation and the consequence of this is the appearance of small muscle fibers with abnormal lipid rafts seen in our patient. Little information is available on how ultrastructure of lipid rafts might change in different defects of PM. Muscle fibres coated by abnormal lipid structures as well as the presence of giant caveolae-like invaginations of PM which are occupied by MLMBs seen in our child may suggest that changes in the composition of PM lipids and caveolae organisation may result from abnormal phospholipids and cholesterol accumulation.

Finally, overexpression or accumulation of aberrant lipids on the surface of muscle fibres may indicate a new myopathy related to lipid raft destabilization.

Acknowledgements

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