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CASE REPORT

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Fatal congenital myopathy with actin filament deposits

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Abstract We present the clinical and morphological findings in a case of progressive congenital myopathy. The symptoms present at birth included severe general muscular hypotonia, diffuse muscular atrophy, arthrogryposis, absence of spontaneous movements, and left ventricular hypertrophy. A biopsy specimen taken from the gastrocnemius muscle when the patient was 2 weeks old revealed deposits which consisted of actin filaments as shown by electron microscopy. The infant was occasionally respirator dependent but was mostly able to breathe unassisted. At the age of 5 months he died of respiratory failure. The actin filament deposits may explain the clinical findings.

Key words Actin · Congenital myopathy · Muscle pathology

Introduction

Congenital myopathies are genetically determined muscular disorders with characteristic structural abnormalities in most cases [6]. Most patients present with hypotonia at birth. The motor deficit is non-progressive or even improves with time, but some children die early from respiratory failure (for review, see [8]). It is not always clear whether the structural changes described are an essential factor in these diseases, and what the presumed pathophysiological mechanism is. Occasionally, patients who obviously suffer from a congenital myopathy do not fit into any of the established disease categories. We now describe the case of a child with an unclassifiable fatal congenital myopathy. The muscle fibers contained masses of 6- to 8-nm-thick filaments instead of myofibrils; the child also suffered from cardiomyopathy.

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Case report

A boy born at term showed severe muscular hypotonia and diffuse atrophy, arthrogryposis, micrognathia, hirsutism, and lower facial nerve palsy. Spontaneous movements were reduced, and the child was intermittently dependent on oxygen. Electrocardiogram and plain radiographs suggested ventricular hypertrophy which was later (at age 5 months) confirmed by ultrasound examination. Electromyography of three muscles revealed reduced recruitment during maximal innervation, shortened motor unit potentials, fibrillation potentials and positive sharp waves, but no polyphasia. Motor and sensory nerve conduction was normal. No chromosomal abnormality was found. The parents were not related, and a 6-year-old sister was healthy. The patient died from respiratory failure after 5 months.

A biopsy specimen was taken from the gastrocnemius muscle at age 2 weeks. An additional muscle sample from the biceps muscle was obtained immediately after death, but consent for a complete autopsy was not obtained. The biopsy sample was divided into bundles which were kept at in situ length; one large bundle was frozen in nitrogen slush and processed for enzyme- and immunohistochemistry; small bundles were fixed with glutaraldehyde and processed for electron microscopy. The muscle sample taken after death was fixed at in situ length with Zamboni's solution (paraformaldehyde-picric acid; [12]). Sections of unfixed material were subjected to a standard battery of enzyme histochemical stains. Immunohistochemistry was done on frozen sections of unfixed and Zamboni-fixed material; the antibodies used were monoclonal anti-vimentin and anti-desmin (Boehringer and Dako, respectively) and the binding sites were labelled with biotinylated anti-mouse immunoglobulin and avidin-horseradish peroxidase (HRP)-diaminobenzidine (DAB). In addition, teased fiber bundles of Zamboni-fixed specimens were stained with anti-vimentin or anti-desmin, the binding sites were labelled with biotinylated anti-mouse and avidin-HRP-DAB, and the fiber bundles were embedded for electron microscopy [2].

Results

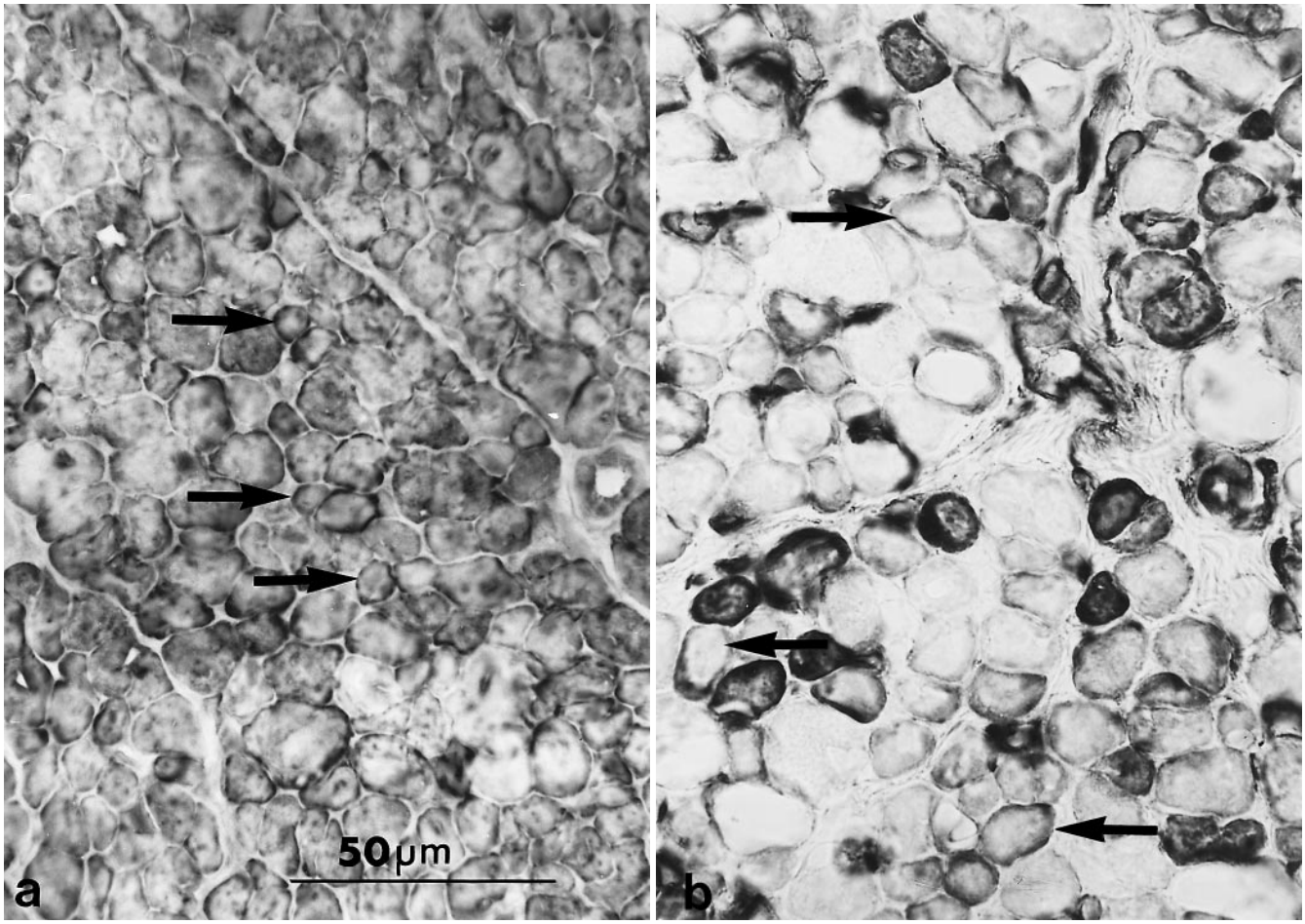
Light microscopy

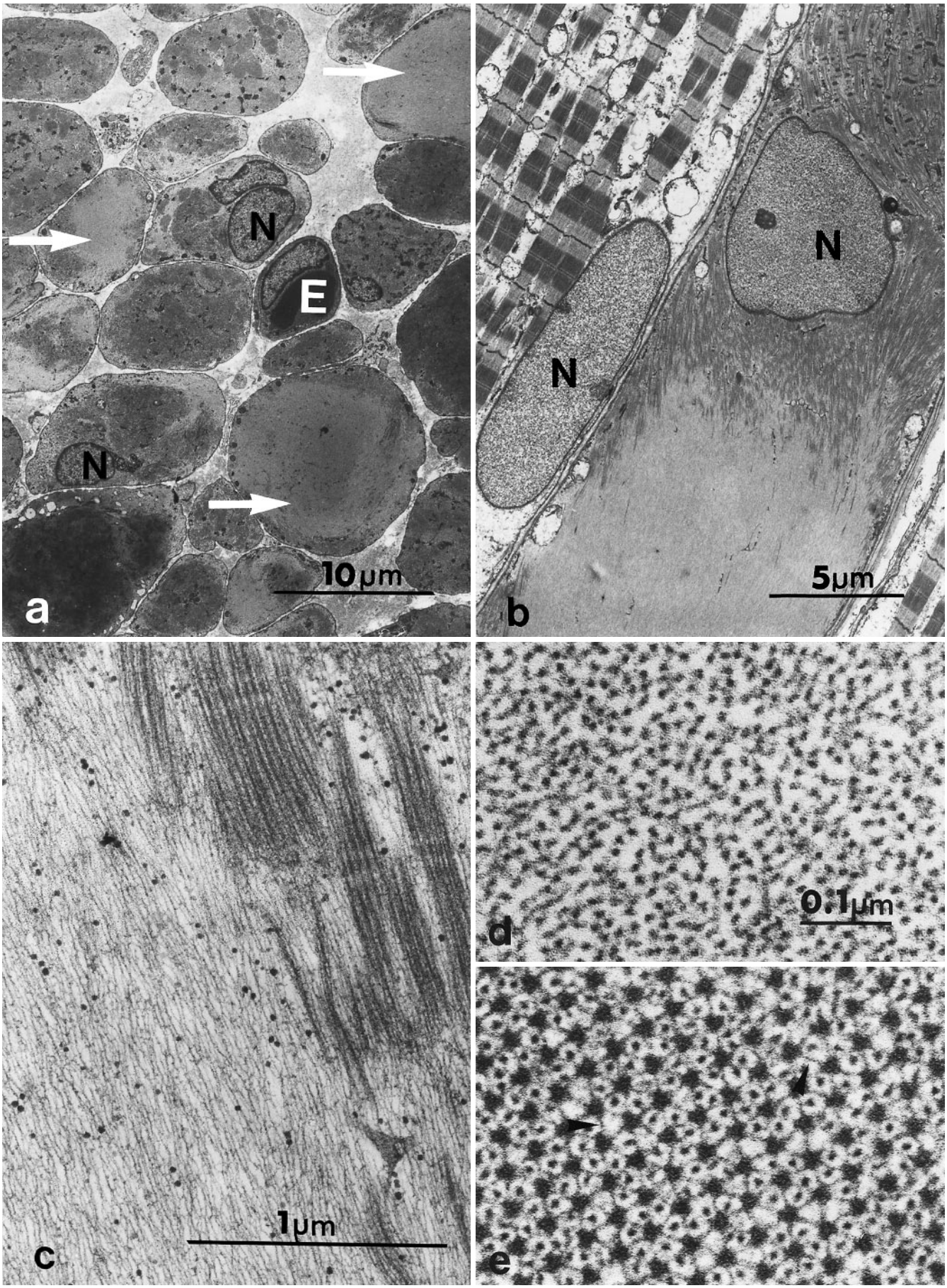
The diameter of the fibers of the gastrocnemius muscle biopsied 2 weeks after birth ranged from 3 to 15 μm (mean 9.3 μm). Some fascicles were dominated by type 1 fibers, which tended to be thicker than type 2 fibers. Type grouping was not seen. Almost half of the fibers on cross sections showed staining defects for mitochondrial enzymes (not shown). The trichrome and ATPase staining appeared normal at first sight. Only when reviewed after the ultrastructure had been examined were violaceous areas noticed with the trichrome stain, and areas of reduced staining intensity seen in the ATPase-stained sections (not shown), both of which corresponded to the unstained areas with the mitochondrial enzyme stains. One third of the fibers stained more intensely than normal for desmin (Fig. 1a) and also for vimentin (Fig. 1b); the remaining fibers reacted with normal intensity for desmin and not for vimentin. The areas devoid of mitochondrial staining failed to react for desmin or for vimentin. In those fibers, desmin and vimentin reactivity was thus limited to a peripheral rim. Periodic acid Schiff staining was normal. The brachial biceps muscle at age 5 months showed pronounced fatty infiltration; the muscle fibers measured 4–20 μm in diameter (mean 9.8 μm). Large intramuscular nerve branches contained normally myelinated nerve fibers (not shown). The mean diameter of fibers of normal vastus muscles at age 0–6 months is 11–14 μm [10].

Fig. 1 a, b *Light microscopy* of biceps brachii muscle. **a** Anti-desmin staining. Note multiple unstained central areas *(arrows)*. **b** Anti-vimentin staining. A proportion of small but also large fibers show abnormal reaction with the antibody (normal muscle fibers would be unstained). As in **a**, the reaction product is usually in the periphery of the fibers and does not selectively stain their center, which contains the filamentous deposits *(arrows)* (see Fig. 2)

Electron microscopy

The specimens obtained 2 weeks and 5 months after birth showed essentially the same changes. The muscle fibers varied considerably in size and were smaller than normal (Fig. 2a). In many places, the normal myofibrils had been replaced by masses of more or less parallel-running filaments 6–8 nm thick (Fig. 2a–c). No remnants of a sarcomere pattern were found in these regions. Transitional zones between normal myofibrils and the thin filaments suggested that the filaments originated from the normal myofilament pattern (Fig. 2b). The filaments within the deposits resembled those actin filaments that formed nor-




N
E
N
10μm
a
N
N
5μm
b
1μm
c
0.1μm
d
e

◀ **Fig. 2 a–e** *Electron micrographs.* **a, b** Cross and longitudinal sections to show the filamentous deposits which sometimes occupy almost the entire cross-sectional area of a fiber (*arrows* in **a**). The fiber diameter varies considerably (**a**). Some fibers still have normal sarcomeres while others contain distorted sarcomeres (**b**) (*E* erythrocyte inside a capillary, *N* myonuclei). **c** Apparent transition between sarcomeres and the filaments forming the filamentous deposits. The dark granules are beta-glycogen. **d, e** High magnification cross sections through a filamentous deposit (**d**) have the same diameter (6–8 nm) as thin actin filaments between thick myosin filaments (**e**). The M-line bridges connecting thick filaments are distinct *(arrowheads)*; not all actin filaments extend into this zone, and the hexagonal filament pattern is incomplete

mal sarcomeres (Fig. 2 c–e) with respect to diameter. One third to one half of the fibers showed deposits of thin filaments which occupied the center and often the entire cross-sectional area of a fiber (Fig. 2 a). Mitochondria, glycogen or intracellular membranes were lacking within the filamentous deposits. In areas which contained neither normal myofibrils nor masses of thin filaments small accumulations of cytoplasmic glycogen were seen, but vacuoles containing glycogen were absent. The crista structure of the mitochondria was normal, and no nemaline bodies, cytoplasmic bodies, or core-like structures could be detected. Myonuclei were normal. The interstitial space contained basal lamina remnants indicating loss of muscle fibers (not shown). Intramuscular nerve branches and blood vessels were normal. The structure of smooth muscle cells in the walls of arteries appeared normal. Sections of fibers stained with anti-desmin or anti-vimentin by a pre-embedding procedure did not reveal staining of the filamentous deposits.

Discussion

The patient described here suffered from a fatal congenital myopathy characterized by large deposits of 6- to 8-nm-thick filaments which resembled actin filaments. Thick filaments or Z-lines were lacking in these regions. The hypertrophic cardiomyopathy in the patient may indicate that the disorder also affected heart muscle cells. Skeletal muscle fibers were hypotrophic, and there was no evidence for a metabolic myopathy or a neurogenic disorder.

The filamentous deposits probably corresponded to the fiber areas that, in frozen sections, did not stain for mitochondrial enzymes. Sections reviewed in the light of the electron microscopic findings also showed fiber areas with reduced staining for ATPase and violaceous areas in the trichrome stain. Nevertheless, the diameter of the fibers (less than 10 μm) was in the range of the thickness of the section, and it was not possible to decide whether the staining defects represented myonuclei, minute freezing artifacts, or myofibrillar abnormalities. It was, thus, not possible to decide the nature of the filamentous deposits on light microscopical grounds.

We initially suspected that the muscle fibers contained excessive amounts of intermediate filaments as in the desmin myopathy described by Edström et al. [7]. Nevertheless, antibodies to desmin or vimentin did not show an abnormal staining pattern in frozen sections, and the filaments were not stained by a pre-embedding immunocytochemical technique which reliably had labelled desmin and vimentin filaments on other material [2]. The staining pattern for desmin and vimentin corresponded to that seen in muscles containing many immature fibers [3]. Electron microscopy suggested that the deposited filaments were actin filaments, but we were unable to confirm this by immunocytochemistry because the amount of muscle tissue that could be studied was limited.

Several authors have described loss of myosin filaments in patients with inherited [1, 9, 13] or acquired myopathy [4, 5, 11]. In all these cases, however, at least a rudimentary sarcomere pattern with smeared Z-lines was preserved. The patient described by Gibbels et al. [9] eventually developed nemaline myopathy. The patient of Bertini et al. [1], an 8-year-old girl who had developed normally, presented with hypertrophic cardiomyopathy which caused death within 3 months. They also found a myopathy with disarrayed myofibrils and loss of thick filaments; atrophic type 1 fibers failed to react for ATPase after preincubation at all pH conditions. A heart muscle biopsy showed cells with focal loss of myofibrils and filamentous deposits resembling those in our patient. Bertini et al. [1] tentatively suggested that their patient had a mutation of the type 1 beta-myosin heavy chain which is expressed both in heart and skeletal muscle. This explanation, which also might explain the disorder in our patient, needs confirmation.

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References

1. Bertini E, Bosman C, Salviati G, et al (1993) Myopathy and hypertrophic cardiomyopathy with selective lysis of thick filaments. Virchows Arch [A] 422 : 327–331
2. Bornemann A, Schmalbruch H (1992) Desmin and vimentin in regenerating muscles. Muscle Nerve 15 : 14–20
3. Bornemann A, Schmalbruch H (1993) Anti-vimentin staining in muscle pathology. Neuropathol Appl Neurobiol 19 : 414–419
4. Carpenter S, Karpati G, Rothman S, Watters G (1976) The childhood type of dermatomyositis. Neurology 26 : 952–962
5. Danon MJ, Carpenter S (1991) Myopathy with thick filament (myosin) loss following prolonged paralysis with vecuronium during steroid treatment. Muscle Nerve 14 : 1131–1139
6. Dubowitz V (1985) Muscle biopsy: a practical approach. Baillière Tindall, London
7. Edström L, Thornell LE, Eriksson A (1980) A new type of hereditary distal (skeletin) filaments. J Neurol Sci 47 : 171–190
8. Fardeau M, Tomé FMS (1994) Congenital myopathies. In: Engel AG, Franzini-Armstrong C (eds) Myology, 2nd edn. McGraw-Hill, New York, pp 1487–1532
9. Gibbels E, Kellermann K, Schädlich HJ, Adams R, Haupt WF (1992) Follow-up studies in a case of unusual congenital myopathy, suggestive of nemaline type. Acta Neuropathol 83 : 371–378

10. Oertel G (1988) Morphometric analysis of normal skeletal muscles in infancy, childhood and adolescence. An autopsy study. J Neurol Sci 88:303–313
11. Sher JH, Shafiq SA, Schutta HS (1979) Acute myopathy with selective lysis of myosin filaments. Neurology 29:100–106
12. Stefani M, DeMartino C, Zamboni L (1967) Fixation of ejaculated spermatozoa for electron microscopy. Nature 216:173–174
13. Yarom R, Shapira Y (1977) Myosin degeneration in a congenital myopathy. Arch Neurol 34:114–115