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Dominantly inherited myopathy with novel tubular aggregates containing 1–21 tubulofilamentous structures

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Abstract Tubular aggregates (TAs) in skeletal muscle fibers have been observed as a nonspecific finding in a number of different conditions such as periodic paralysis, myotonic disorders, hyperaldosteronism, chronic use of drugs, and alcoholism. However, TAs were also found more specifically in well-defined muscle disorders, e.g., exercise-induced cramps, myasthenic syndromes, and even in dominantly or recessively inherited familial myopathies. We report on a presumably dominantly inherited familial myopathy with late onset characterized morphologically by the presence of three types of TAs in type II muscle fibers identified in three affected members of one family (a 86-year-old man and his two sons). The first, novel type was characterized by tubules, 30–200 nm in thickness which included 1–21 tubulofilamentous structures 14–18 nm in diameter. The second type of TAs corresponded to previously well-described tubules and were derived from terminal cisternae, which were rather irregularly arranged or widened, and filled with material of medium electron density. The third type of TAs were occasional, hexagonally arranged TAs of the usual type [type Ib and Ic according to [24]. Rare annulate lamellae were also seen. Our findings support the evidence of tubular aggregates as the major finding in certain dominantly inherited myopathies. Tubules of the first type, to the best of our knowledge, have not been recorded in any other myopathy. It is therefore suggested that these tubules characterize a novel type of a benign, slowly progressive myopathy with late onset, muscle pain, cramps, and stiffness.

Keywords Tubular aggregates · Dominantly inherited familial myopathy · Cramps · Muscle pain · Stiffness

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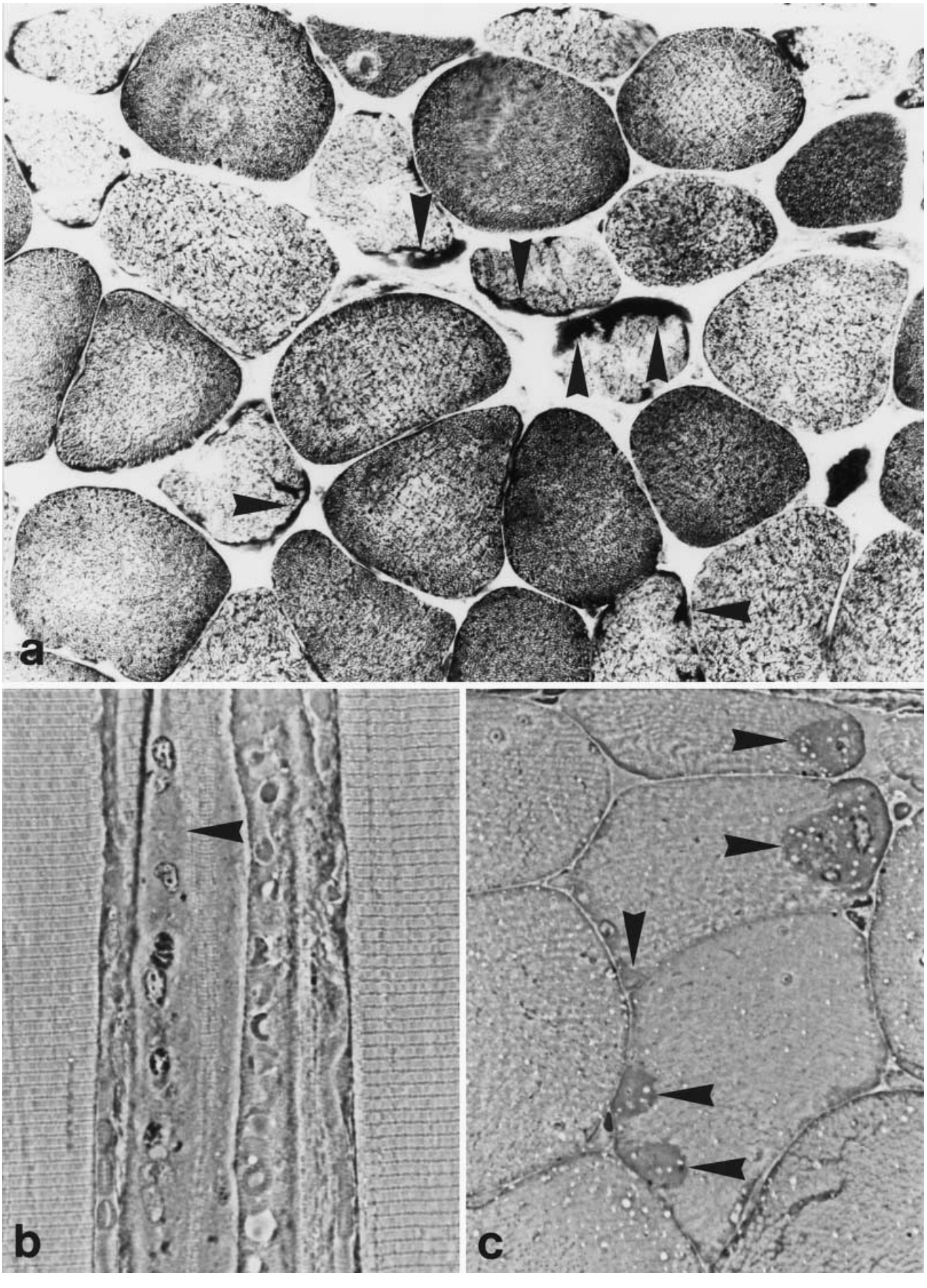
Introduction

Tubular aggregates (TAs) have been found in a variety of skeletal muscle disorders. They have been observed as a nonspecific finding in myasthenia gravis [4], inflammatory myopathies [12], myotonia [23], and alcohol- and drug-induced myopathies [5, 7]. TAs are found most characteristically in a group of patients with exertional muscle pain, stiffness, and cramping, and in cases of periodic paralysis [2, 13, 20], but have also been reported in dominantly [3, 18, 21, 22] or recessively [6] inherited familial myopathies with TAs.

TAs are structures of variable appearance. They are usually composed of closely packed double-walled tubules often containing a single inner tubular structure or amorphous material [24]. TAs are usually present in type II muscle fibers, but are occasionally also found in type I fibers. Recent experimental work confirmed that TAs originate from the sarcoplasmic reticulum (SR) [23].

We report on an presumably dominantly inherited myopathy (father more severely affected than the two sons) characterized by various TAs, the first a novel type which includes 1–21 tubulofilamentous structures not previously seen in other well-known types of TAs (type I–IV according to Schröder and Becker [24]). Clinical and histochemical details concerning these cases will be described separately [25]. The present study focuses on the fine structural aspects of the various TAs seen in these three cases of one family.


a
b
c

◀**Fig. 1a–c** Case 1. **a** Cryostat section showing strong NADH-TR staining of the TAs (*arrowheads*). **b, c** Longitudinal and transverse semithin sections. The TAs are lying predominantly in a subsarcolemmal position (*arrowheads*). There are some nuclei between the moderately stained TAs (*TAs* tubular aggregates). **a** ×260; **b, c** ×840

Case reports

Four family members in three generations were investigated. The main clinical findings are summarized here. Further details of the clinical and immunohistochemical data in each individual patient will be presented in a separate study [25].

Case 1

This patient (II-1) was seen at the age of 53 years and presented with progressive muscle weakness. He had some difficulty in climbing stairs. He developed a tendency to fall, finding it very difficult to get up by himself and had been receiving retirement pay for 1 year. There was moderate paresis of the neck flexors, finger extensors, and hip flexors. Gower's sign was positive. Standing on the heels was difficult. Other important complaints were exertional stiffness particularly concerning the fingers. Furthermore, the patient complained of myalgia and back pain due to muscle stiffness. He had suffered from cramps in the legs at night for 20 years.

Case 2

This patient (II-2) is the 48-year-old brother of patient II-1, who had complained of myotonia-like symptoms with stiffness of the hands especially when performing fine motor tasks for the previous 5 years. He suffered from slowly progressive muscle fatigue, acute pain lasting for seconds only in his arms and legs, and cramps in the legs. He stumbled often like his brother, but did not fall and showed mild paresis on clinical examination.

Creatine kinase (Ck) was mildly raised up to 4.5 μmol/s per l (normal values: <3.1 μmol/s per l) in patients 1 and 2, and EMG showed a myopathic pattern with pseudomyotonic discharges.

Case 3

This patient (I-1) was the father of patients II-1 and II-2 and wheelchair dependent for the last 20 years. First symptoms were noted at the age of about 45 years. Muscle pain and slowly progressive weakness had been noted, and he had fallen very often. He died at the age of 86 of pneumonia and was autopsied.

Case 4

This patient (III-2) is the 29-year-old son of patient II-2. He and his younger brother were clinically asymptomatic until now.

Methods

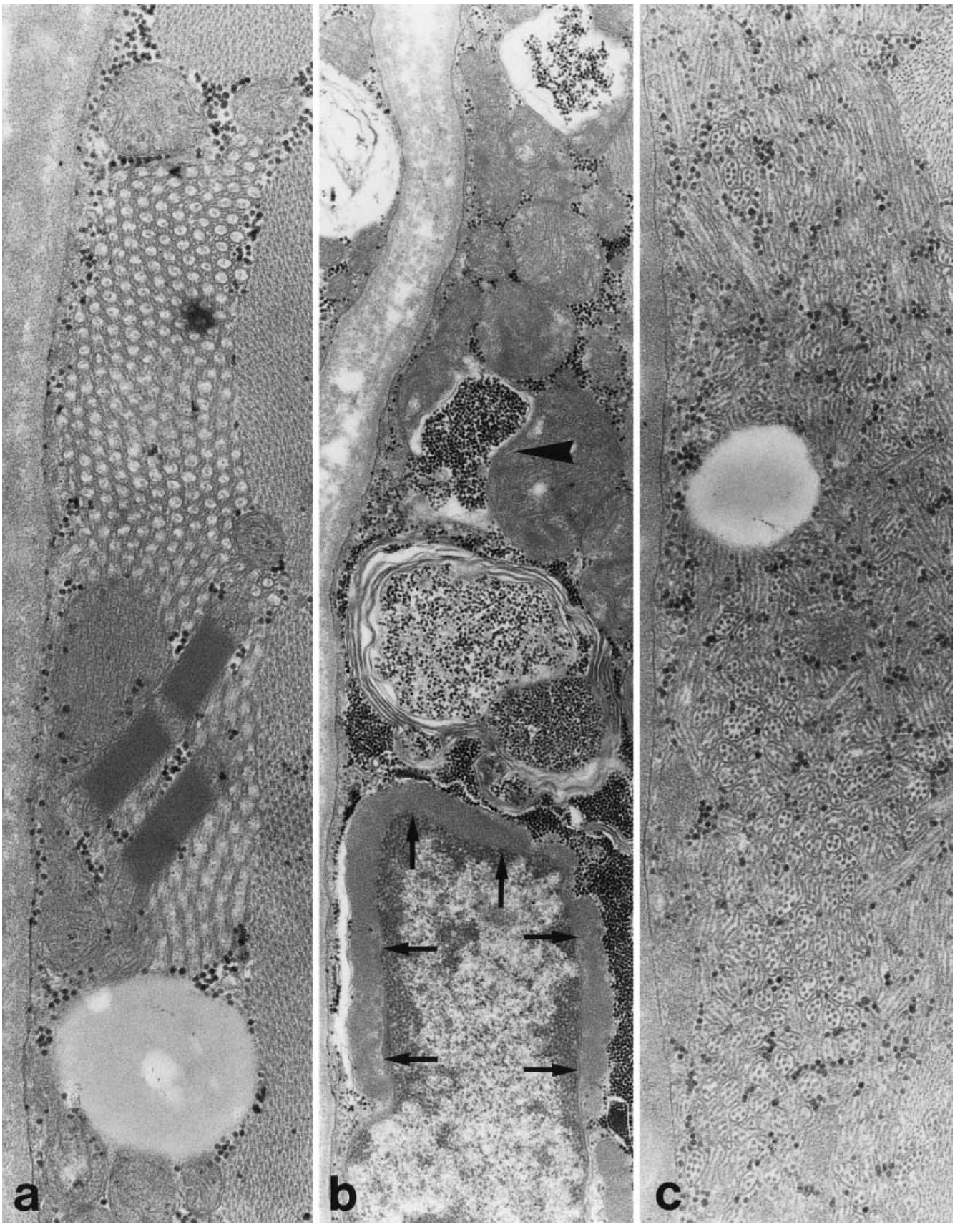
Muscle biopsy specimens were taken from the deltoid muscles in cases 1, 2 and 4. For paraffin embedding, samples of muscle were fixed in formaldehyde. For semithin sections and electron microscopy, segments of muscle were fixed in 6% glutaraldehyde with 0.1 M phosphate buffer, pH 7.4. For histochemistry, segments of the deltoid muscle biopsy specimen were immediately frozen in liquid nitrogen and stored at –80°C until processing. Cryostat sections were stained with hematoxylin and eosin, methylene blue, modified Gomori's trichrome, periodic acid-Schiff (PAS), and oil-red O. Myofibrillar ATPase after preincubation at pH 9.4, 4.6, and 4.2, NADH-TR, acid phosphatase, succinic dehydrogenase (SDH), and cytochrome *c* oxidase (COX) reactions were also performed. Part of the muscle biopsy sample was used to prepare semithin sections, which were stained with paraphenylenediamine for light microscopy. Ultrathin sections were contrast enhanced with uranyl acetate and lead citrate, and examined with a Philips T400 electron microscope. Autopsy specimens of the father's (case 3) deltoid muscle were processed as in his sons. General autopsy was not permitted by the relatives.

Results

All investigated muscle biopsy specimens showed mainly normal fiber diameters ranging from 40 to 80 μm with increased variation in fiber size. The muscle biopsy samples of patients II-1 and II-2 showed pronounced type II fiber atrophy. There was predominance of type I fibers. Fiber type grouping was absent. In the muscle biopsy specimens of these two patients about one third of type II fibers in the H&E stains showed multiple basophilic, subsarcolemmal, or centrally located substances. These regions stained red with the modified Gomori's trichrome and reacted intensively blue with the NADH-tetrazolium reduc-

Table 1 Different forms of tubules in tubular aggregates. Supplemented classification of Schröder and Becker [24] in comparison to the classification of Cameron et al. [3]

Type		Outer diameter of tubules	Contents	Type according to [3]
Ia	Proliferated terminal cisternae	80–90 nm	Homogeneous or flocculent electron dense material	–
Ib	Tubular aggregates	50–80 nm	Central tubule or a microtubule-like structure	I
Ic	Tubular aggregates	40–50 (–400) nm	"Empty" or moderately dense, flocculent material	II
Id	Dilated parallel tubules	130–400 nm	Microtubule-like structures, about 25–40 nm in diameter	III
Ie	Present type of tubular aggregate	30–200 nm	1–21 tubulofilamentous structures, about 14–18 nm in diameter	–
IIa	Tubules with filaments	90–140 nm	Granular	–
IIb	Giant tubules with filaments	200–250 nm	"Empty"	–


a
b
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◀ **Fig. 2a–c** Case 1. **a** There are hexagonally arranged tubular structures with adjacent filaments (similar to type IIa in Table 1, although smaller with diameters of 40–50 nm). Two mitochondria surrounded by TAs contain paracrystalline inclusions. **b** The fibrous lamina of the nucleus is thickened (*arrows*). Enlarged mitochondria are partially filled with glycogen granules (*arrowhead*). Other glycogen granules are surrounded by membranous lamellae. **c** TAs (type Ie in Table 1), 35–200 nm in diameter including up to 15 tubulofilamentous structures in this plane of section. **a** ×44,800; **b** ×26,000; **c** ×45,500

tase (Fig. 1a). The TAs were predominantly located in a subsarcolemmal position (Fig. 1b, c). The adenosine triphosphate reaction at pH 9.4 or after preincubation at pH 4.6, and 4.2 did not stain these accumulations. Also, no staining with PAS or oil-red O was observed. The samples did not display activity for SDH and COX. The muscle biopsy specimens of patient III-2 revealed only minor abnormalities. There was only a slightly extended spectrum of muscle fiber diameters. Some central nuclei and a minor increase of predominantly subsarcolemmal glycogen was also apparent. No cellular infiltrates or signs of muscle fiber necrosis or myophagic reactions were seen in any of the muscles examined.

Electron microscopic examination of the muscles of the patients I-1, II-1, and II-2 showed large accumulations of subsarcolemmal and to a lesser degree intermyofibrillar aggregates of unusual tubules (type Ie in Table 1), which were arranged in varying directions. These tubules consisted of a single dense wall and usually included one or two, or more rarely 3 to 21 tubulofilamentous structures measuring about 14–18 nm in diameter (Figs. 2c, 3a–c). These inclusions within the tubules appeared to be slightly thicker and of moderately higher contrast and sharper contour than adjacent myosin filaments (which are known to measure 16 nm in diameter). They were usually compact or filamentous in cross sections, but some filaments showed a light center, suggesting a basically tubular substructure. In longitudinal sections, an uneven periodicity of their contour was noted, showing fine indentations with a periodicity of approximately 20 nm. The outer diameter of the (outer) tubules in transverse sections varied from 30 to 200 nm depending on the number of tubulofilamentous inclusions. The outer tubular membrane did not differ in thickness or contrast when compared to the surface membrane of muscle fibers. Some other aggregates contained only tubules with a small number of inner tubulofilamentous structures (mostly 1 or 2) and only a few larger tubules containing up to 5 tubulofilamentous inclusions (Figs. 4a, 5a). These tubules were oriented in large or small groups in parallel or at random. Between the tubules there were loosely disseminated glycogen granules. Other TAs of this type were characterized by predominantly large tubules containing more than 10 inner tubulofilamentous structures; they were arranged irregularly or in rows. The closest distance of the inner structures between each other as well as to the outer wall was constantly about 10 nm. These unusual TAs were usually closely packed; sparse glycogen granules or even lipid droplets surrounded the individual tubules within the TAs.

A second type of tubules (type Ia in Table 1) which were derived from terminal cisternae of the SR were rather irregularly arranged or widened, and filled with more or less electron-dense material (Figs. 3e, 4b–d, 5b). In addition, there were occasional hexagonally arranged TAs consisting of parallel tubular arrays of the usual type Ib or Ic (Table 1) containing moderately flocculent material or suggestive inner tubules with a larger diameter than those in the new type Ie in Table 1 (Fig. 2a).

Some mitochondria were increased in size and contained paracrystalline inclusions (Fig. 2a). Focally, the myofibrils surrounding the TAs showed nonspecific pathological changes. Additionally there were occasionally small clusters of annulate lamellae (Fig. 3f), a few filamentous bodies, and an unusual thickening of the fibrous lamina of a nucleus (Fig. 2b).

Electron microscopic examination of the left deltoid muscle of the case III-2 showed a slightly increased number of mitochondria surrounded by lipid droplets and glycogen granules. There were also some filamentous bodies in a subsarcolemmal position. Only one small aggregate of eight evenly proliferated terminal cisternae (type Ia in Table 1) was found (not shown). The novel TAs with tubulofilamentous structures seen in the deltoid muscles of the other three patients were not detectable in patient III-2 who showed no clinical symptoms of a myopathy.

Discussion

Unusual TAs were noted in the present study consisting of tubules which include 1–21 tubulofilamentous structures. The diameter of the (outer) tubules varied between 30 and 200 nm depending on the number of inner tubulofilamentous structures which measured 14–18 nm in diameter. This type of TA was classified as type Ie according to the present classification (Table 1) and differed from the well-known tubules described in previous studies [3, 24].

A second type of TAs (type Ia in Table 1), derived from terminal cisternae, were irregularly arranged and filled with material of medium electron density. As a third type, there were occasional hexagonally arranged TAs (type Ib and Ic in Table 1) consisting of parallel tubular arrays of the usual type.

TAs corresponding to type Ie (in Table 1), to the best of our knowledge, have not been recorded in any other myopathy, although other types of TAs have been described in numerous neuromuscular disorders, including periodic paralysis [19], myasthenic syndromes [4, 8, 9], inflammatory myopathies [12], myotonia [24], and in many nonspecific muscle disorders associated with pain and stiffness or exercise-induced muscle cramps [2, 10, 16, 20]. Aggregates have also been noted in muscle in association with alcohol and drug abuse [5, 7]. Therefore, some authors have considered TAs as a nonspecific phenomenon without clinical significance or that they may represent an adaptive response [14, 18].

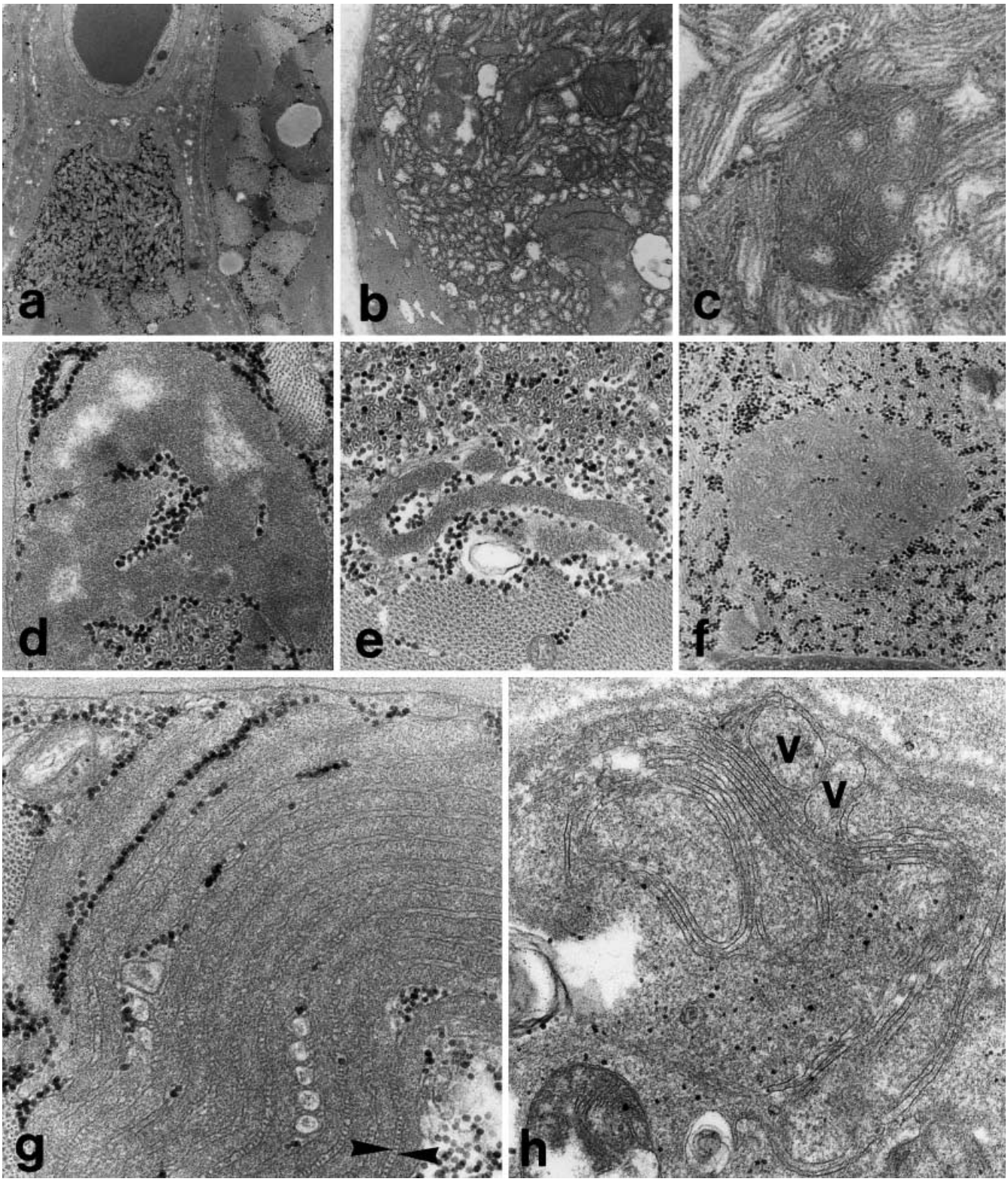

Fig. 3a–h Case 1. **a–c** Electron micrographs showing TAs of the novel type (type Ie in Table 1) with variable numbers and orientation at different magnifications. There are glycogen granules and abnormal mitochondria adjacent to or between the TAs. **d** Amorphous material of medium electron density of enlarged terminal cisternae is seen lying between glycogen granules, TAs and myofibrils. **e** Isolated tubules derived from terminal cisternae (type Ia in Table 1) are filled with material of medium electron density between TAs of type Ie and myofibrils. **f** Annulate lamellae are apparent between TAs and glycogen granules. **g** Tortuous arrangement of terminal cisternae showing intermediate lines or dotted structures with a diameter of approximately 3–5 nm (*arrowheads*). **h** Small subsarcolemmal vacuoles (*V*) are associated with what appear to be elongated tubules filled with amorphous material or showing an internal line of moderate electron density. **a** ×6,600; **b** ×12,000; **c** ×51,000; **d** ×39,000; **e** ×36,400; **f** ×25,000; **g** ×49,300; **h** ×40,300

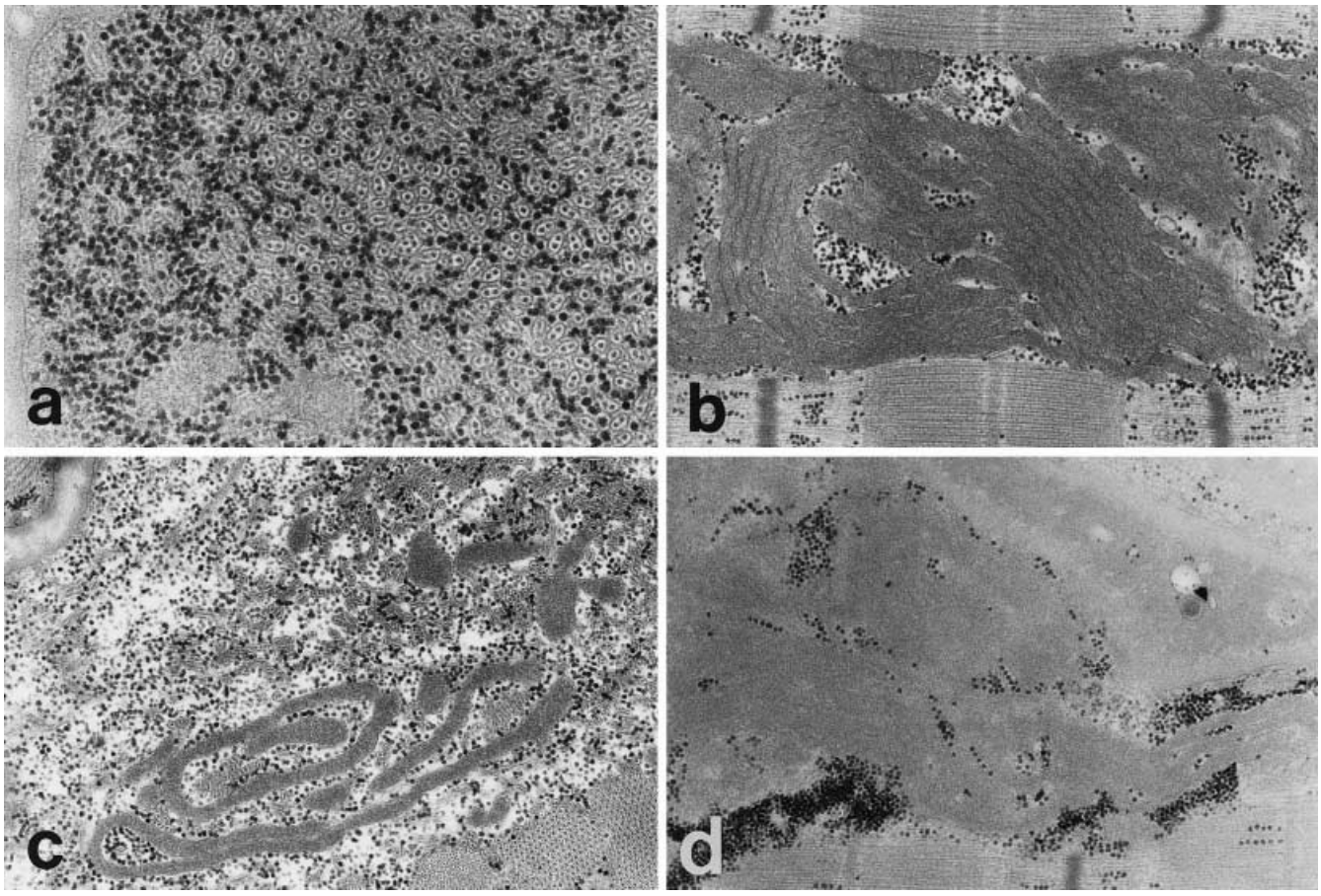

Fig. 4a–d Case 2. **a** Cluster of TAs (type Ie in Table 1) showing single, or more rarely, several tubulofilamentous inclusions are surrounded by glycogen granules. **b–d** Tubules derived from terminal cisternae (type Ia in Table 1) are rather irregularly arranged or widened and filled with material of medium electron density. **a** ×45,500; **b** ×25,900; **c** ×23,000; **d** ×24,000

Until now there were only six other case reports describing familial myopathies with TAs. In five of these families there was presumably dominant inheritance [3, 18, 21, 22], while in one case [6] only two sibs were affected. Other cases with a familial myopathy were reported but in no case were TAs documented in the muscle biopsy specimens of more than one family member [1, 8, 9, 13].

The TAs of the previous reported, dominantly inherited ‘myopathies with TAs’ varied in their ultrastructural appearance. They were usually located in a subsarcolemmal or intermyofibrillar position and were arranged in closely packed clusters with hexagonal patterns in cross-sections embedded in glycogen granules, and sometimes adjacent to lipid droplets or lipofuscin granules. These tubules mostly corresponded to type I or type II of Cameron et al. [3], or type Ib or type Ic of Schröder and Becker [24], respectively. The average diameter of the outer membrane of the double-walled tubules was about 60–80 nm containing a smaller central tubule with an average diameter of about 20–40 nm. The single membranous tubules containing electron-dense flocculent material (type II of Cameron et al. [3] or type Ic of Schröder and Becker [24]) were more variable in size with an average diameter of 10–400 nm.

The unusual tubular structures (type Ie) in our patients resembled to a certain degree type III tubular structures described by Cameron et al. [3]; however, there were substantial differences. First, type III tubules according the classification of Cameron et al. [3] consisted of dilated parallel tubules mostly between 130 and 400 nm in diameter containing smaller, 25- to 40-nm-diameter microtubule-like structures. The type Ie tubules seen in our cases were usually smaller in outer diameter with an broader range. Second, while the inner microtubule-like structures shown by Cameron et al. [3] were relatively large showing an unequivocal tubular appearance, the TAs noted in our cases contained inner structures with a strikingly variable number (1–21), and a smaller diameter (15–18 nm).

The clinicopathological significance of TAs is still unsettled. TAs have been found predominantly or almost exclusively in type II fibers [1, 6, 10, 15, 18, 19], but were also described in type I fibers [8, 21, 22]. The origin of TAs from the SR membrane is widely accepted and disturbances of the calcium homeostasis by TAs have been discussed [23, 25]. Other authors proposed that TAs may be represent an unspecific adaptive response of the SR. There is also immunohistological evidence suggesting the involvement of heat shock protein in the formation of TAs

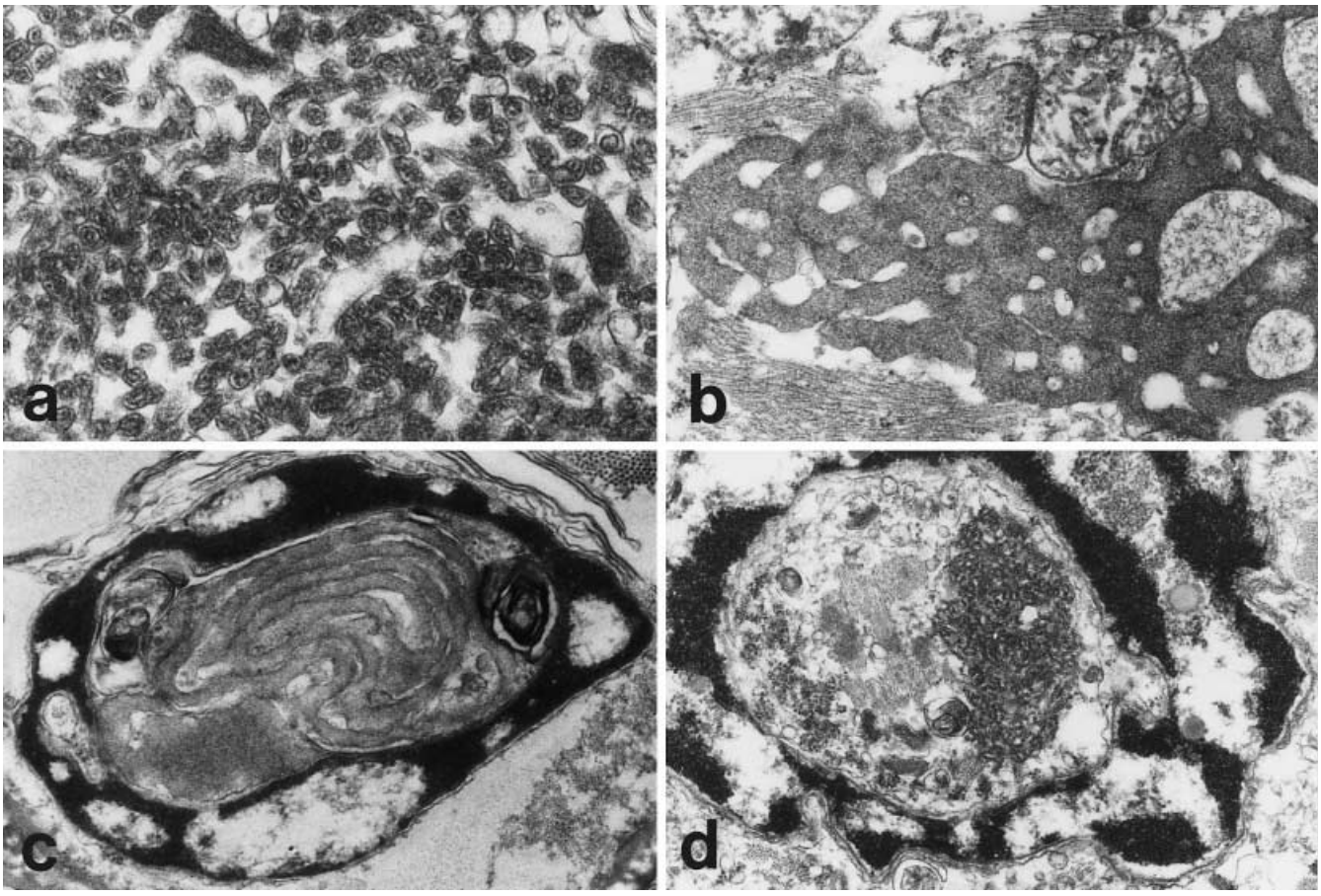

Fig. 5a–d Case 3 at autopsy. **a** Aggregates of novel tubules (type Ie in Table 1) containing a variable number of tubulofilamentous structures which are well preserved in spite of advanced autolysis. **b** Adjacent to some mitochondria there are tortuous tubules with electron-dense material which appear to be connected with each other (type Ia in Table 1). **c** Sarcoplasmic invagination of a nucleus showing proliferated terminal cisternae (type Ia in Table 1). **d** Sarcoplasmic invagination of a nucleus with degraded myofibrillar, granular, and tubulovesicular structures. **a** ×41,600; **b** ×25,000; **c** ×23,200; **d** ×19,200

by causing conformational changes in the structure of the proteins of the SR [17].

Despite the unusual ultrastructural appearance of the TAs noted in the present cases, it is very likely that they are also derived from the SR as in the other cases with dominantly inherited TAs, in which a remarkable variability of the ultrastructural appearance was described, whereas the histochemical features were uniform with strong reactions for NADH-tetrazolium reductase and myoadenylate deaminase as well as bright red staining with the modified Gomori's trichrome method and basophilic appearance with the H&E stain.

In conclusion, the present study revealed a novel type of TAs as the major finding in a benign, probably dominantly inherited myopathy clinically characterized by late onset, slowly progressive muscle weakness with muscle pain, cramps, and stiffness.

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