

Morphological spectrum and clinical features of myopathies with tubular aggregates

Fabian Funk^{1,2,3}, Chantal Ceuterick-de Groote⁴, Jean-Jacques Martin^{4,5}, Axel Meinhardt¹, Ana L. Taratuto⁶, Jan De Bleecker⁷, Rudy Van Coster⁸, Boel De Paepe⁸, Ulrike Schara⁹, Matthias Vorgerd¹⁰, Martin Häusler¹¹, Stefan Koppi¹², Matthias Maschke¹³, Peter De Jonghe⁵, Lionel Van Maldergem¹⁴, Stéphane Noel¹⁵, Christoph W. Zimmermann¹⁶, Stefan Wirth¹⁷, Stefan Isenmann¹⁸, Rudolf Stadler¹⁹, J. Michael Schröder^{1,3}, Jörg B. Schulz^{2,3}, Joachim Weis^{1,3} and Kristl G. Claeys^{1,2,3}

¹Institute of Neuropathology, RWTH Aachen University, Aachen, Germany, ²Department of Neurology, RWTH Aachen University, Aachen, Germany, ³JARA Translational Brain Medicine, ⁴Laboratory of Ultrastructural Neuropathology, Institute Born-Bunge, University of Antwerpen, Antwerpen, Belgium, ⁵Department of Neurology, University Hospital Antwerpen, Antwerpen, Belgium, ⁶Institute for Neurological Research, FLENI, Buenos Aires, Argentina, ⁷Department of Neurology, University Hospital Gent, Gent, Belgium, ⁸Department of Pediatrics, Division of Pediatric Neurology and Metabolism, University Hospital Gent, Gent, Belgium, ⁹Department of Pediatric Neurology, University of Essen, Essen, Germany, ¹⁰Department of Neurology, University Hospital Bergmannsheil, Ruhr University Bochum, Bochum, Germany, ¹¹Department of Neuropediatrics, RWTH Aachen University, Aachen, Germany, ¹²Department of Neurology, Regional Hospital of Rankweil 1, Rankweil, Austria, ¹³Department of Neurology and Neurophysiology, Hospital Barmherzige Brüder, Trier, Germany, ¹⁴Centre de Génétique Mumaïne, Université de Franche-Comté, Besançon, France, ¹⁵Department of Neurology, University Hospital, Charleroi, Belgium, ¹⁶Department of Neurology, St. Josef Hospital/Academic Teaching Hospital of the University of Essen, Oberhausen, Germany, ¹⁷Children's Hospital, Helios Klinikum Wuppertal, Wuppertal, Germany, ¹⁸Department of Neurology, Helios Klinikum Wuppertal, Wuppertal and University of Witten/Herdecke, Germany and ¹⁹Department of Dermatology, Johannes-Wesling-Klinik, Minden, Germany

Summary. Tubular aggregates (TAs) are aggregates of densely packed tubules in human skeletal muscle fibers with particular histochemical and ultrastructural features that most probably arise from the sarcoplasmic reticulum. Some studies have shown an additional mitochondrial origin of TAs. We studied the histopathological spectrum and clinical features in a large cohort of patients with TAs in their muscle biopsy (106 biopsies), derived from our muscle biopsy archive (15,412 biopsies in total). In particular, we examined light microscopic, enzyme histochemical, immunohistochemical and ultrastructural features in the muscle biopsies, as well as the patients' clinical data. We found TAs in 0.5% of all muscle biopsies. Based on the size of TAs, we identified two sub-groups: (1) myopathies with large TAs (29 biopsies) in type 2 fibers and sometimes also in type 1 fibers, absence of any other associated disorder, and a familial history in half of the cases, and (2) myopathies with small TAs (77 biopsies), exclusively

in type 2 fibers, presence of another associated disease in the majority of patients and mostly no familial history. In the sub-group with large TAs, we observed a high variability of ultrastructural changes. The most frequent clinical symptom in both groups was limb muscle weakness. No significant differences in clinical presentation, age at onset or disease duration at the time of biopsy were found between the two groups. In conclusion, myopathies with TAs can be sub-divided into a group with large TAs, probably corresponding to the so-called primary TA myopathies, and into a group with small TAs as a feature of another underlying condition.

Key words: Tubular aggregate myopathy, TAM, Skeletal muscle, Ultrastructure, Sarcoplasmic reticulum

Introduction

In 1964, Engel described aggregates in human skeletal muscle fibers with certain histochemical features, such as an intense reaction for reduced nicotinamide-adenine-dinucleotide dehydrogenase

(NADH), staining by modified Gomori's trichrome (GT) and absence of succinate dehydrogenase (SDH) (Engel, 1964). The aggregates were named tubular aggregates (TAs) after additional ultrastructural analysis was performed in 1970 (Engel et al., 1970). TAs typically appear as a group of tubules oriented in parallel, with diameters ranging from 40-200 nm at electron microscopic (EM) level, however, a large variety of ultrastructural features has been described (Engel et al., 1970; Schröder and Becker, 1972; Cameron et al., 1992; Pavlovicová et al., 2003). A number of different TA classifications have been proposed based on ultrastructural findings (Schröder and Becker, 1972; Cameron et al., 1992; Pavlovicová et al., 2003). The derivation of TAs from the sarcoplasmic reticulum (SR) was shown by EM and immunohistochemical studies (Engel et al., 1970; Pierobon-Bormioli et al., 1985; Salviati et al., 1985; Chevessier et al., 2005). Other enzyme histochemical and immunohistochemical studies suggest that TAs may arise from both SR and mitochondria (Lewis et al., 1971; Rosenberg et al., 1985; Novotova et al., 2002).

Familial and sporadic cases of tubular aggregate myopathies (TAM) with TAs as the predominant histopathological finding have been reported (Engel et al., 1970; Lewis et al., 1971; Schröder and Becker, 1972; Dobkin and Verity, 1978; Sipilä et al., 1979; Rohkamm et al., 1983; Pierobon-Bormioli, 1985; Rosenberg et al., 1985; Salviati et al., 1985; Cameron et al., 1992; Sieb et al., 1996; Furui et al., 1997; Martin et al., 1997; Alonso-Losada et al., 1998; Müller et al., 2001; Jacques et al., 2002; Sharizaila et al., 2004; Chevessier et al., 2005; Ghosh et al., 2010; Guerguelcheva et al., 2011; Senderek et al., 2011). For most cases of familial TAM, an autosomal-dominant inheritance has been suggested (Rohkamm et al., 1983; Cameron et al., 1992; Martin et al., 1997; Müller et al., 2001; Shahrizaila et al., 2004). Furthermore, various, occasional associations of TAs with other hereditary myopathies, such as myotonic myopathy (Schröder and Adams, 1968; Schröder and Becker, 1972; Rosenberg et al., 1985), congenital myasthenic syndromes (Guerguelcheva et al., 2011; Belaya et al., 2012; Huh et al., 2012) or acquired systemic conditions such as alcoholic myopathy (Gullotta and Helpap, 1975; del Villar Negro et al., 1982) are known.

Major clinical phenotypes of myopathies with TAs involve exertional myalgia, muscle cramps and stiffness, with or without weakness (Pierobon-Bormioli, 1985; Rosenberg et al., 1985; Martin et al., 1997; Müller et al., 2001), slowly progressive proximal weakness (Rohkamm et al., 1983; Alonso-Losada et al., 1998), limb-girdle myasthenia with predominant fatigability (Dobkin and Verity, 1978; Sieb et al., 1996; Furui et al., 1997; Guerguelcheva et al., 2011; Senderek et al., 2011) and periodic paralysis (Engel et al., 1970; Jurkat-Rott et al., 2000; Luan et al., 2009).

In the present study, we characterized the histopathological spectrum and clinical features in a

large cohort of 93 index patients (i.e. unrelated patients) with TAs in their muscle biopsy (106 biopsies in total), in order to identify morphological-clinical sub-groups that might be helpful in orienting further diagnosis.

Materials and methods

Biopsy retrieval and selection

We screened the database of the muscle tissue archive of the Institute of Neuropathology, University Hospital RWTH Aachen, Germany, for muscle biopsies containing TAs. A total of 15,412 muscle biopsies obtained within the period from 1981 to 2011 were analyzed for the search terms "tubular" and/or "aggregates". In our study, we included biopsies of individuals who showed characteristic features of TAs at the light microscopic or EM level according to Cameron and colleagues (Cameron et al., 1992). Additional muscle biopsies showing TAs were provided by the Laboratory of Ultrastructural Neuropathology, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium (20 biopsies), the Department of Neurology, University Hospital Gent, Gent, Belgium (3 biopsies) and the Institute for Neurological Research, FLENI, Buenos Aires, Argentina (1 biopsy). Studies on family 1 (Fig. 1; Martin et al., 1997) and family 2 (Fig. 1; Müller et al., 2001) have been published previously. Diagnostic open skeletal muscle biopsies were performed. The muscles that were used for biopsy are indicated in Table 1. This study was approved by the ethical committees of the different cooperating centers and was conducted according to the Declaration of Helsinki.

Patients, clinical and paraclinical data

For all patients included in the present study, we retrospectively collected information about the age and symptoms at onset, presence of weakness, periodic paralysis, myalgia, fatigability, cramps, stiffness, contractures and myoglobinuria. Medical history and drug use were considered, as well as the family history. A general clinical and neurological examination was performed. We also evaluated available information about neurophysiological results (EMG/NCV/repetitive nerve stimulation), imaging (muscle/brain), laboratory values (CK/lactate), cardiologic data (ECG/echocardiography/Holter-ECG), respiratory tests (lung function/vital capacity), and diagnostics on tissue samples.

Light microscopy and enzyme histochemistry

Muscle biopsy specimens were processed using standard histological techniques (Dubowitz and Sewry, 2007). For histological studies, 10µm thick frozen sections of unfixed material were stained using the following reactions: hematoxylin eosin (HE), modified Gomori's trichrome (GT), periodic acid Schiff (PAS),

Pathological spectrum in TA myopathies

oil-red-O (ORO), adenylate deaminase (AMPD), NADH-tetrazolium reductase (NADH-TR), succinate dehydrogenase (SDH), cytochrome oxidase (COX) and adenosine triphosphatase (ATPase) at pH 9.4, 4.6 and 4.2. We reviewed all available reactions of the biopsies for features of TAs. The percentage of fibers containing TAs and the size of the TAs was measured following AMPD reaction using an Axioskop microscope (Carl Zeiss AG, Oberkochen, Germany). The fiber type of muscle fibers containing TAs was assessed using ATPase reactions at pH 9.4, 4.6 and 4.2. Other features examined were fiber type predominance, myopathic and neurogenic changes, and the presence of necrotic and regenerating fibers, vacuoles, ragged red fibers (in GT staining) and COX-negative/SDH-positive fibers.

Immunohistochemistry

In 6 biopsies (I.I.1, I.II.1, I.III.1, 1004, 1017, 1018; Table 1; Fig. 1), we additionally evaluated the immunohistochemistry of the TAs. Normal, control samples were included in the immunohistochemical analyses. Antibodies included in the study were directed against: alpha-actinin (mouse; monoclonal-anti-human, clone BM-75.2; 1:500; Sigma), alpha-B-crystallin (rabbit; polyclonal-anti-human; Novocastra), alpha-dystroglycan (mouse; ascites IgM, clone I1H6C4; 1:1000; Upstate biotechnology), alpha-sarcoglycan (50kD; mouse; 1:200; Dr. K. Campbell), alpha-tubulin (mouse; 1:1000; Sigma), Alzheimer precursor protein, 22c11 (mouse; 1:10; Boehringer), ApoE (goat; 1:2000; Leiden), beta-amyloid-protein-sequence-2-43,A4 (rabbit; 1:500; Dr.K.Beyreuther), beta-dystroglycan (43kD; mouse; 1:200; Novocastra), caveolin-3 (goat; 1:1000; SantaCruz), desmin (mouse; 1:200; Dakopatts),

developmental myosin-heavy-chain (MHC-d; mouse; 1:50; Novocastra), dysferlin (mouse; 1:1000; Novocastra), dystrophin-1 (mouse; monoclonal-anti-human-rod-domain; 1:5; Novocastra), dystrophin-2 (mouse; monoclonal-anti-human C-terminus; 1:5; Novocastra), dystrophin-3 (mouse; monoclonal-anti-human N-terminus; 1:100; Novocastra), dystrophin-associated glycoprotein (43kD and 50kD) (mouse; 1:200; Novocastra), emerin (mouse; 1:100; Novocastra), fast MHC (MHC-f; mouse; 1:200; Novocastra), HLA-ABC (mouse; 1:200; Dakopatts), HLA-DR (mouse; 1:50; Dakopatts), laminin (mouse; anti-human; clone 4C7; 1:100; Dakopatts), merosin (mouse; monoclonal-anti-human merosin 80kD; 1:2000; Chemicon + mouse; monoclonal-anti-human merosin 300kD; Novocastra), myosin (rabbit; monoclonal-anti-human fast-skeletal-myosin, clone MY-32; 1:50; Sigma), myotilin (mouse; 1:1000; Novocastra), nebulin (mouse; 1:800; Sigma), sarcomeric actin (mouse; monoclonal-anti-human muscle actin, HHF35; 1:1000; Dako), slow MHC (MHC-s; mouse; 1:200; Novocastra), spectrin (mouse; 1:500; Novocastra), SR-Ca²⁺-ATPase-1 (mouse; 1:500; Novocastra), SR-Ca²⁺-ATPase-2 (mouse; 1:500; Novocastra), tau AT-8 (mouse; 1:20,000; Innogenetics), tau AT-120 (mouse; 1:5000; Innogenetics), telethonin (goat; 1:1000; SantaCruz), tropomyosin (mouse; 1:400; Sigma), troponin-T (mouse; 1:500; Amersham), ubiquitin (rabbit; 1:500; Dakopatts), utrophin (mouse; 1:100; Dr. G.E. Morris), and vimentin (mouse; 1:200; Dakopatts). The Novocastra antibodies are now marketed by Leica and the Chemicon antibodies by Millipore. Additionally, we have used in one biopsy with SDH-positive large TAs an antibody against SDH (more precisely against the IP subunit of complex II; mouse; 1:4; MitoSciences). For immunolabeling the 3,3'-

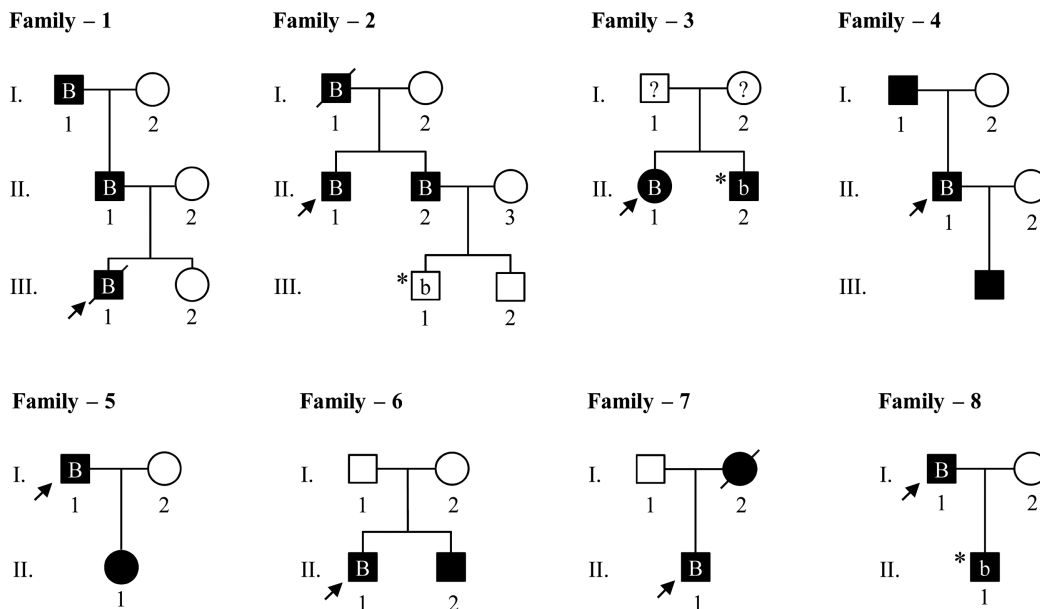


Fig. 1. Pedigrees of familial cases with TAs. Black boxes indicate affected individuals (clinical symptoms and/or CK elevation). Question mark (?), unknown clinical status. B, Biopsy with TAs; b, biopsy without TAs. The arrow points to the index patients. Asterisks indicate the biopsies of three relatives that were additionally included in the present study.

diaminobenzidine technique was used.

Ultrastructural studies

For EM, muscle samples were fixed in phosphate-buffered 4% glutaraldehyde, osmicated in 1% phosphate-buffered osmium tetroxide, dehydrated and embedded in Epon. Semithin sections (1–2 μm) were stained by paraphenylene diamine and methylene blue. The ultrathin sections (95 nm) of at least one transverse and one longitudinal block were incubated in the contrast agents, uranyl acetate and lead citrate. Electronmicrographs were taken using a CM10 transmission EM (Philips, Amsterdam, Netherlands) with the integrated sheet-film camera. We evaluated position, type and size of the TAs, further abnormalities of the SR and of connected membranous systems, the presence of honeycombs, mitochondrial abnormalities, amount of glycogen and lipids, myofibrillar structure, nuclear abnormalities and the presence of autophagic vacuoles.

Measurement of the TAs

We measured the surface area of the largest visible TAs in transverse EM images using ImageJ 1.44p software (Wayne Rasband, National Institutes of Health, Bethesda, Maryland, USA) and in transverse light-microscopic sections using the AxioCam camera (Carl Zeiss AG, Oberkochen, Germany) and the AxioVision software (Carl Zeiss AG, Oberkochen, Germany). The largest surface area of both measurements was further used.

Classification of the tubular aggregates

We classified the TAs according to Cameron and co-workers (Cameron et al., 1992) and to Pavlovicová and colleagues (Pavlovicová et al., 2003). Cameron and colleagues defined aggregates of tubules, each approximately 70 nm in diameter with a smaller inner tubule or microtubule-like structure (type I; Fig. 2B), aggregates of tubules each measuring between 70 and 400 nm in diameter and containing moderately dense, flocculent material (type II; Fig. 2E), and aggregates of dilated tubules each mostly between 130 and 400 nm in diameter and containing a number of microtubule-like structures (type III; Fig. 2C, indicated with an asterisk).

In addition to these three TA types, Pavlovicová et al. (2003) defined TAs with filamentous tubules (FTTA; Fig. 2F,G), corresponding to type IIa of Schröder and Becker (Schröder and Becker, 1972), aggregates with tubulofilamentous structures (TFTA; Fig. 2I,J) which correspond to the novel type of TAs first described by Müller et al. (Müller et al., 2001), vesicular membrane collections (VMC, Fig. 2E), proliferated terminal cisterns (PTCs) that can be sub-divided into regularly (RPTC; Fig. 2K) or irregularly (IPTC; Fig. 2L) PTCs that had been shown by Schröder et al. (Schröder and

Adams, 1968; Schröder and Becker, 1972). We also screened the biopsies for vacuoles most likely derived from SR and/or transverse tubuli (Shy et al., 1961; Dubowitz and Sewry, 2007; Fig. 2H), and giant tubules with a diameter of 200 to 250 nm (Schröder and Becker, 1972). Although sub-sarcolemmal membranous structures (SMS; Gullotta and Helpap, 1975; Reske-Nielsen et al., 1975; Fig. 2M) differ substantially from TAs by origin and shape, we additionally screened the biopsies for the presence of SMS.

Results

Frequency and size of TAs

In our muscle biopsy archive, which comprises 15,412 muscle biopsies, we identified 82 biopsies belonging to 79 individuals (76 index patients, i.e. unrelated patients) that contained TAs, corresponding to a frequency of 0.5%. Additionally, we included 24 biopsies from 23 patients (17 index patients) with TAs obtained from other centers. Thus, our study comprises 106 biopsies from 103 patients (93 index patients) in total.

In each biopsy, we measured the size of the largest TA at transverse sections (Fig. 3; Table 1: size listed for myopathies with large TAs). An arbitrarily chosen cut-off value of 125 μm^2 for the TA surface area was used. Even if a biopsy contained mostly small TAs and more rarely large TAs, the size of the largest TA was measured and if the surface area was above 125 μm^2 , the biopsy was classified as myopathy with large TAs (example in Fig. 4J). A TA surface area of at least 125 μm^2 was present in 29 biopsies, whereas in the other 77 biopsies the size of the largest TA ranged from 0.1 to 124 μm^2 (Fig. 3). In each transversely cut light microscopic section with visible TAs, we counted on average 683 muscle fibers (range 194–1029) in a randomly selected position to determine the percentage of muscle fibers containing TAs. We found a large variability in frequency of TAs ranging from 1 to 90% (Table 1: frequency listed for myopathies with large TAs). Sometimes a focal distribution of affected fibers was noticed. There was no correlation between the size and the frequency of TAs in the muscle biopsies, which might be due to mixing data from various muscles that differ in the prevalent fiber type. In a further study, we explored in detail 33 biopsies belonging to 25 index patients with TAs of at least 125 μm^2 . The muscle biopsies of three patients (Fig. 1, asterisks) were additionally included for further study because they were related to one of the index patients.

Light microscopic and enzyme histochemical features of biopsies with TAs

The light microscopic features of the 33 biopsies with TAs larger than 125 μm^2 are summarized in the Table 1. In all cases, TAs showed a positive reaction for

Pathological spectrum in TA myopathies

Table 1. Maximal surface area and electron-microscopic features in myopathies with large tubular aggregates.

Ind.	Muscle	Sarcoplasmic Reticulum										Metabolism				Other features					
		Tubular aggregates			Additional features							Mitochondria				Myofibrils		Nuclei			
		Max. surf. (μm^2)	Tub. diamtr. (nm)	Local.	I	II	III	Ptc	Vac.	SMS	Hon. cmb.	↑ No.	Crst. abnl.	Swell. / deg.	Incl.	↑ Gln.	↑ Lip.	Dis- Org.	Z str.	Abnl. Int.	Aut. vac.
1.III.1	VL	2200	48-355	S, I, N	++	+	+	-	+	-	±	-	+	-	-	±	-	-	+	+	-
1.II.1	VL	3900	49-276	S, I, N	++	+	+	-	++	-	+	-	-	-	-	-	+	-	-	+	+
1.I.1	D	600	49-190	S, I, N	++	+	+	-	+	-	+	-	+	-	±	±	+	-	-	+	+
2.II.1	D	1600	55-235	S, I, N	+	-	++	rptc	+	+	-	-	-	+	-	-	-	-	+	+	-
					ftta		ftta	iptc													
2.II.2	NA	850	30-135	S, I, N	+	+	++	-	±	-	-	-	±	-	-	-	-	-	-	±	-
							tfta														
2.III.1	NA	-	-	-	-	-	-	rptc	-	-	-	-	-	-	-	-	-	-	-	-	-
2.I.1	NA	-	-	-	-	-	-	iptc	-	+	-	-	+	±	-	±	+	-	+	-	-
3.II.1	VL	1700		S, I, N	+	+	+	-	+	-	-	-	±	-	-	-	+	-	-	+	±
					ftta	ftta															
3.II.2	NA	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	±	-	±	-	±
4.II.1	G	287	>60	S, I	+	-	+	-	++	-	+	-	+	+	-	-	++	+	+	+	±
5.I.1	BB	3100	35	S, I, N	+	+	-	-	++	-	-	-	+	-	-	-	-	-	-	±	-
6.II.1	NA	340	50;	S	+	vmc	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
			240-350																		
7.II.1	RF	> 490	90-110	S, I, N	-	+	-	rptc	+	-	-	±	-	-	-	-	-	-	+	-	-
								iptc													
8.I.1	Q	-	no EM																		
8.II.1	Q	125	no EM																		
9*	TA	1650	61-290	S, I, N	+	+	-	-	+	-	-	-	-	-	-	++	-	+	+	+	-
9*	NA			no EM																	
15	BB	800	-	-	-	-	-	-	-	+	-	-	-	±	-	±	-	-	+	-	-
19	Q	1250	80	S, I, N	+	±	±	-	++	-	-	-	-	-	-	-	-	-	-	-	-
22	NA	300	45-55	S, I, N	+	+	-	rptc	+	-	-	-	-	±	-	-	-	-	+	-	-
					ftta																
32	Q	2250	120-200	S, I, N	-	+	-	-	+	+	-	-	-	±	-	-	-	-	-	+	-
					ftta																
42	BB	2800	55	S, N	+	-	-	-	-	-	-	-	-	-	±	+	+	-	±	-	-
52	G	1300	90-200	S, I, N	-	+	-	-	+	-	-	-	-	-	±	±	-	-	-	-	-
66	TB	340	60 low	S, I, N	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-
70	BB	1500	50;	S, I, N	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-
			30-170			vmc															
75	NA	437	55	S, I, N	+	-	-	rptc	+	-	-	-	+	-	-	-	±	±	-	+	-
84	G	217	65	S, N	+	-	-	rptc	-	-	-	-	+	±	-	-	-	-	-	-	-
89	VL	300	40-160	S, I, N	+	+	-	-	-	-	-	-	±	+	±	-	-	-	-	-	-
					ftta	vmc															
1004	G	2500	48-114	S, I, N	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-
1017*	Q	2800	50-60;	S, I, N	+	+	±	-	+	-	-	-	-	-	-	-	-	-	-	+	-
			33-220																		
1017*	G	650	>50	S, I, N	+	±	+	-	+	-	-	-	-	-	-	-	±	-	-	+	-
2001	NA	1300	no EM																		
3001	BB	4900	90	S	-	+	-	-	-	-	-	-	±	±	-	-	-	-	-	-	-
4001	D	360	50-200	S, I, N	+	+	-	-	+	-	-	-	-	±	-	-	-	-	-	-	-
						vmc															

++, Feature considerably present; +, feature present; ±, feature present in part; -, feature absent; *, two biopsies performed in the indicated person. Ind., number of the individual; muscle, name of the muscle used for biopsy; max. surf., surface area of the largest tubular aggregate in transversal section; tub. diamtr., range of the diameter of the tubules; local., localization of the TAs; I / II / III, types of TAs; dil. trd., dilated triads; ptc, proliferated terminal cisterns; Vac., vacuoles derived from the sarcoplasmic reticulum and/or from transverse tubules; SMS, subsarcolemmal membranous structures; hon. cmb., honey combs; ↑No., increased number; crst. abnl., abnormalities of the cristae; swell. / deg., swelling / degeneration; incl., inclusions; ↑Gln., increased amount of glycogen; ↑lip., increased amount of lipid droplets; disorg., disorganization; z str., z band streaming; abnl., abnormal; int., internal; aut. vac., autophagic vacuoles. AF, M. adductor femoris; BB, M. biceps brachii; D, M. deltoideus; G, M. gastrocnemius; NA, not available; Q, M. quadriceps femoris; RF, M. rectus femoris; TB, M. triceps brachii; VL, M. vastus lateralis. S, subsarcolemmal; I, intermyofibrillar; N, near nuclei; ftta, TAs with filamentous tubules; tfta, aggregates of tubulo-filamentous structures; rptc, regularly proliferated terminal cisterns; iptc, irregularly proliferated terminal cisterns; vmc, vesicular membrane collections; no EM, no electron microscopy was performed on this biopsy. For abbreviations see further in methods section.

AMPD and NADH-TR. Most TAs stained strongly with the PAS reaction (22/26, 85%), but did not react with the menadione staining. The enzyme-histochemical reactions in biopsies with small TAs did not differ from the biopsies with large TAs. Interestingly, in the three biopsies of family 1 and in the biopsies of 6 index

patients (Table 1 and Fig. 1: 1.I.1, 1.II.1, 1.III.1; 5.I.1, 3, 19, 70, 1004, 1017), the TAs reacted strongly with SDH (Fig. 4H). In contrast, in the other patients as well as in the cases with small TAs (n=77), TAs did not react with SDH. For further results on SDH we refer to the next section on immunohistochemical features.

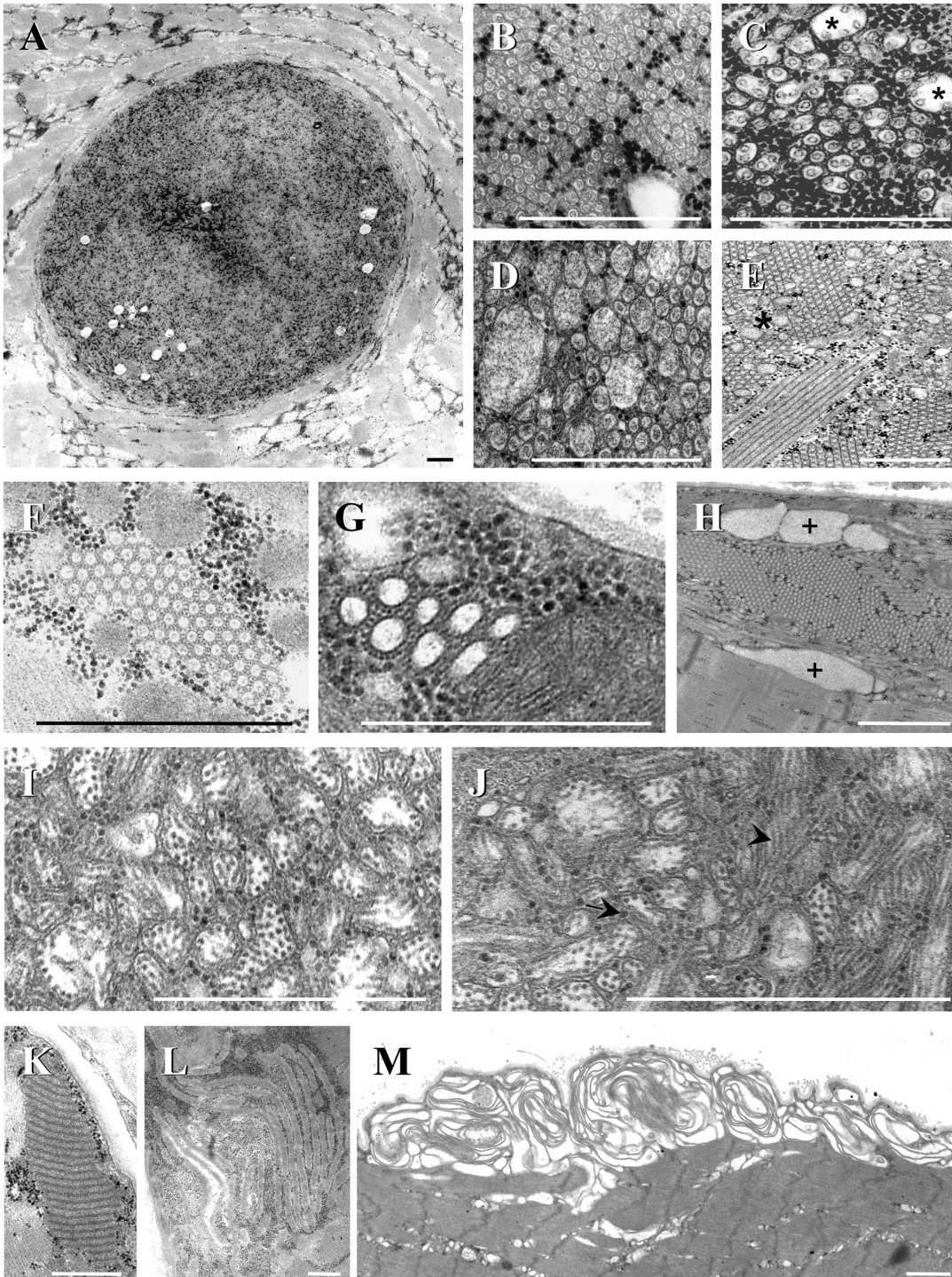


Fig. 2. Ultrastructural spectrum of TAs. **A.** A muscle fiber with a roundly configured TA showing a surface area of $165\mu\text{m}^2$ (patient 1.II.1; Table 1) located between myofibrils in a transversely cut fiber. **B.** Type I tubules, each containing one smaller tubule. **C.** Type I and type III tubules (indicated by asterisks) are shown in the biopsy of patient 1004 (Table 1). **D.** Type I tubules and some small mitochondria (patient 3.II.2; Table 1). **E.** Transversely cut type II tubules arranged in a hexagonal pattern, some longitudinally cut tubuli and VMCs, indicated with an asterisk (patient 70, Table 1). **F.** FTFA with type I tubules. **G.** FTFA with type II tubules. **H.** large vacuoles most likely originating from transverse tubules (+) near a TA (patient 5.I.1, Table 1). **I.** TFTA, the novel type of TAs (patient 2.II.1, Table 1). **J.** TFTA in longitudinal view, showing the presence of multiple inner tubular structures (arrowhead). Glycogen granules are more electrondense and located inbetween the TAs (arrow). **K.** RPTC. **L.** IPTC. Subsarcolemmal membranous structures (SMS) are shown in **M.** Abbreviations: TA, tubular aggregate; VMC, vesicular membrane collections; FTFA, TAs with filamentous tubules; SR, sarcoplasmic reticulum; TFTA, tubulofilamentous TAs; RPTC, regularly proliferated terminal cisterns; IPTC, irregularly proliferated terminal cisterns; SMS, subsarcolemmal membranous structure (for further description see methods section). Scale bars: $1\mu\text{m}$.

In most biopsies, TAs were present in subsarcolemmal regions. In some cases TAs comprised large rounded areas within the muscle fibers, and in others TAs presented as multiple, smaller structures, scattered across the muscle fibers (Fig. 4K). In 10 of 22 biopsies, TAs were present in both type 1 and type 2 fibers, and half of these biopsies belonged to cases with a family history. Nevertheless, the TAs were always more frequent and/or larger in type 2 fibers. In contrast, in the group with small TAs (77 biopsies), which were visible by light microscopy, TAs were exclusively found in type 2 fibers. Pathological predominance of type 1 fibers was present in 4/23 biopsies (17%) and pathological predominance of type 2 fibers was present in 1/23 biopsies (4%). Most biopsies with TAs showed myopathic changes (17/26, 65%), some showed neurogenic alterations (8/26, 31%), necrotic and/or regenerative fibers (4/25, 16%) or vacuoles (2/26, 8%).

Immunohistochemical features of biopsies with TAs

In six biopsies with large TAs, we additionally performed immunohistochemical reactions with antibodies directed against different components of the muscle fiber (Fig. 5). Immunohistochemical data on biopsies with small TAs were not available. TAs showed a positive immunoreactivity for antibodies directed against SERCA-1 (Fig. 5B). In addition, the three biopsies of family 1 revealed SERCA-2 immunoreactive

TAs in both type 1 and type 2 fibers. Thus, labeling of TAs by SERCA was mostly, but not always, of the same isoform as in the rest of the fiber. In the biopsies of patients 1004 and 1017, emerin-positive TAs were noted (Fig. 5F). Single biopsies also showed TAs to be immuno-positive for tau (patient 1.I.1), HLA-ABC (patient 1.II.1), ubiquitin (patient 1017, Fig. 5L), alpha-dystroglycan and telethonin (patient 1004). In the biopsy of patient 1.III.1, TAs were immuno-positive for sarcomeric actin.

In order to exclude the possibility of an unspecific SDH reaction in the SDH-positive TAs, an antibody against SDH (more precisely against the IP sub-unit of complex II) was used in one biopsy with SDH-positive large TAs. The TAs showed a positive reaction for the anti-SDH/IP-antibody, indicating the presence of at least this complex II component in the TAs.

TAs were immuno-negative for alpha-actinin and alphaB-crystallin, ApoE, APP (22C11), AT8, caveolin-3, desmin, dysferlin, dystrophin-1, dystrophin-2, dystrophin 3, dystrophin-associated glycoprotein (43kD and 50kD), fast skeletal myosin, HLA-DR, laminin, merosin, MHC-d, MHC-f, MHC-s, myotilin, nebulin, spectrin, tropomyosin, troponin-T, tubulin, utrophin, vimentin.. The different components of the muscle fibers showed an otherwise normal immunoreactivity for the range of antibodies used.

Ultrastructural spectrum of TAs

Detailed ultrastructural analysis of the sub-group with large TAs is presented in Table 1. We found a large variability of ultrastructural changes (Fig. 2). In the group with large TAs, we found 5/30 biopsies (17%) exclusively showing tubules containing an inner central tubule (type I; Fig. 2B) and 4/30 (13%) exclusively presenting tubules with content of low electron density (type II; Fig. 2E). No biopsy showed exclusive occurrence of an outer tubule with at least two inner tubules (type III; Fig. 2C, asterisks). In 57% of all biopsies, more than one type of TA was present: type I and II in 6/30 biopsies (20%), type I and III in 2/30 biopsies (7%) and all three types in 9/30 biopsies (30%). Interestingly, in one family (family 2, Fig. 1), type III TAs with up to 38 tubules were observed (TFTA, Fig. 2I, 2J; Table 1). We additionally observed FTTA in 5/30 biopsies (17%; Fig. 2F, 2G), VMC in 4/30 biopsies (13%; Fig. 2E) and PTC in 7/30 biopsies (23%; Fig. 2K, 2L). Vacuoles derived from SR and/or from transverse tubuli could be seen in 20/30 biopsies (67%; Fig. 2H). SMS, which differ from rimmed vacuoles by the fact that they do not contain debris, were found in 4/30 biopsies (13%; Fig. 2M). Furthermore, the presence of SMS did not correspond to the presence of rimmed vacuoles at the LM level. At the ultrastructural level, we found no correlate for the SDH-positive reaction of TAs observed in some biopsies, in particular no increase of mitochondria number or of abnormal mitochondria could be found.

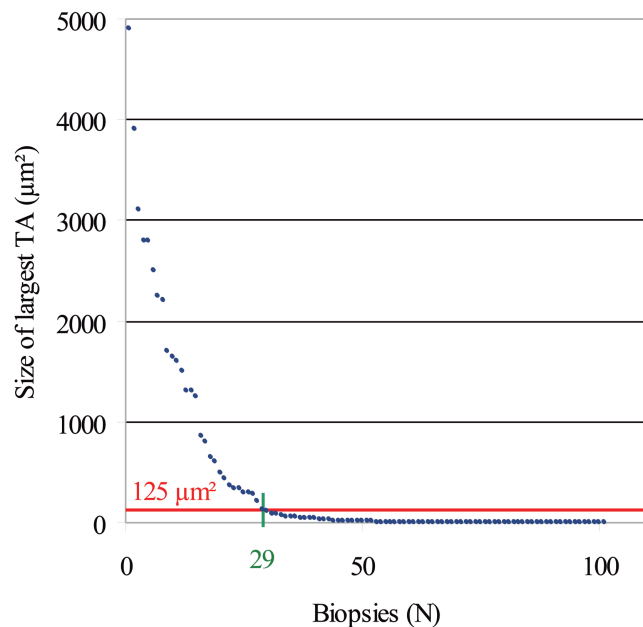


Fig. 3. Size distribution of TAs. Each dot represents the surface area (μm^2) of the largest TA in each muscle biopsy ($n=102$). The cut-off value at $125 \mu\text{m}^2$ results in 29 biopsies with TAs of at least $125 \mu\text{m}^2$ in surface area. The biopsies are arranged according to the size of the largest TAs.

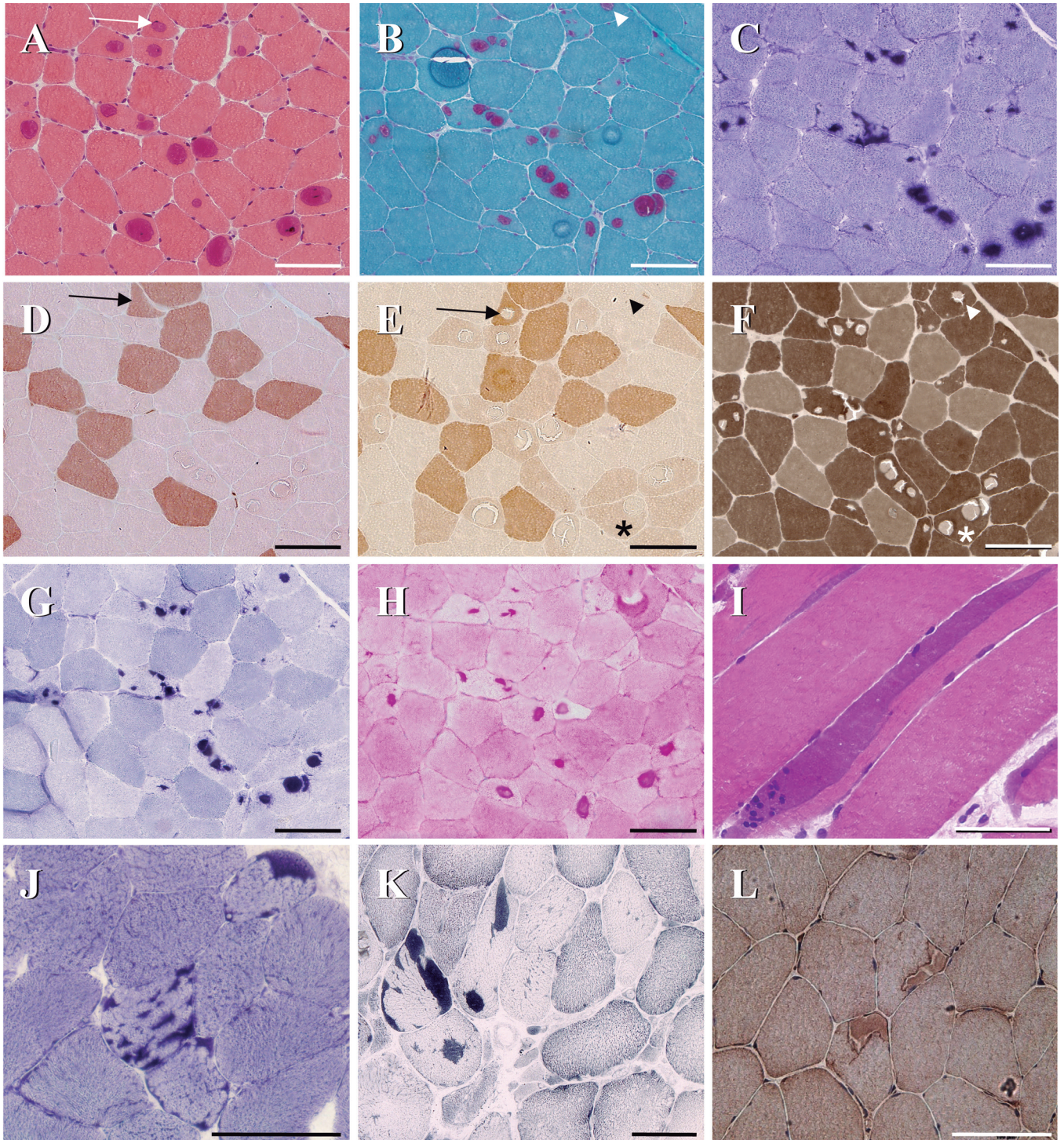


Fig 4. Light microscopic and enzyme histochemical features of TAs. **A-H.** Serial sections of the first muscle biopsy (M. quadriceps) of patient 1017 (Table 1) with large, mostly rounded TAs. **A.** HE; **B.** GT; **C.** AMPD; **D.** ATPase at pH 4.2; **E.** ATPase at pH 4.6; **F.** ATPase at pH 9.4; **G.** NADH-TR; **H.** PAS. TAs show a positive reaction for AMPD, NADH, SDH (**K**) and PAS. In this biopsy most TAs are present in type 2b fibers, which are stained intermediately in ATPase at pH 4.6 and dark in ATPase at pH 9.4 (one exemplary fiber marked with asterisk). Less frequently, TAs can be seen inside type 2a fibers (arrowhead in GT, ATPase at pH 4.6 and 9.2). Rarely, TAs are localized inside type 1 fibers (arrow in HE, ATPase at pH 4.2 and 4.6). **I.** HE: Longitudinal section from the same biopsy, showing a large TA including several myonuclei. **J.** AMPD: Biopsy from the same patient, retrieved one year later (M. gastrocnemius). Here, TAs are multiple, smaller structures and scattered across the muscle fibers. **K.** SDH and **L.** anti-SDH-antibody in biopsy of patient 1.II.1 (Table 1) with TAs in type 2 fibers showing positive reaction for SDH and positive immunoreactivity for an anti-SDH-antibody. Scale bars: 100 μ m.

Pathological spectrum in TA myopathies

Clinical findings

The median age at onset was 37.6 years (range 3-78) in the group with large TAs and 36.4 years (range 2-73) in the group with small TAs. The average disease duration at the time of biopsy was 3.9 years in the first group (range 0-40) and 4.2 years in the second group (0-52). In the group with large TAs, 90% of the index

patients were male, compared to 71% in the other group. The symptoms of patients with large and small TAs are summarized in Table 2. The most frequent symptom in both groups was limb muscle weakness, followed by myalgia, fatigability, muscle cramps and stiffness. No significant differences in clinical presentation were found between the two groups.

In 8 out of 25 index patients (32%) with large TAs, a

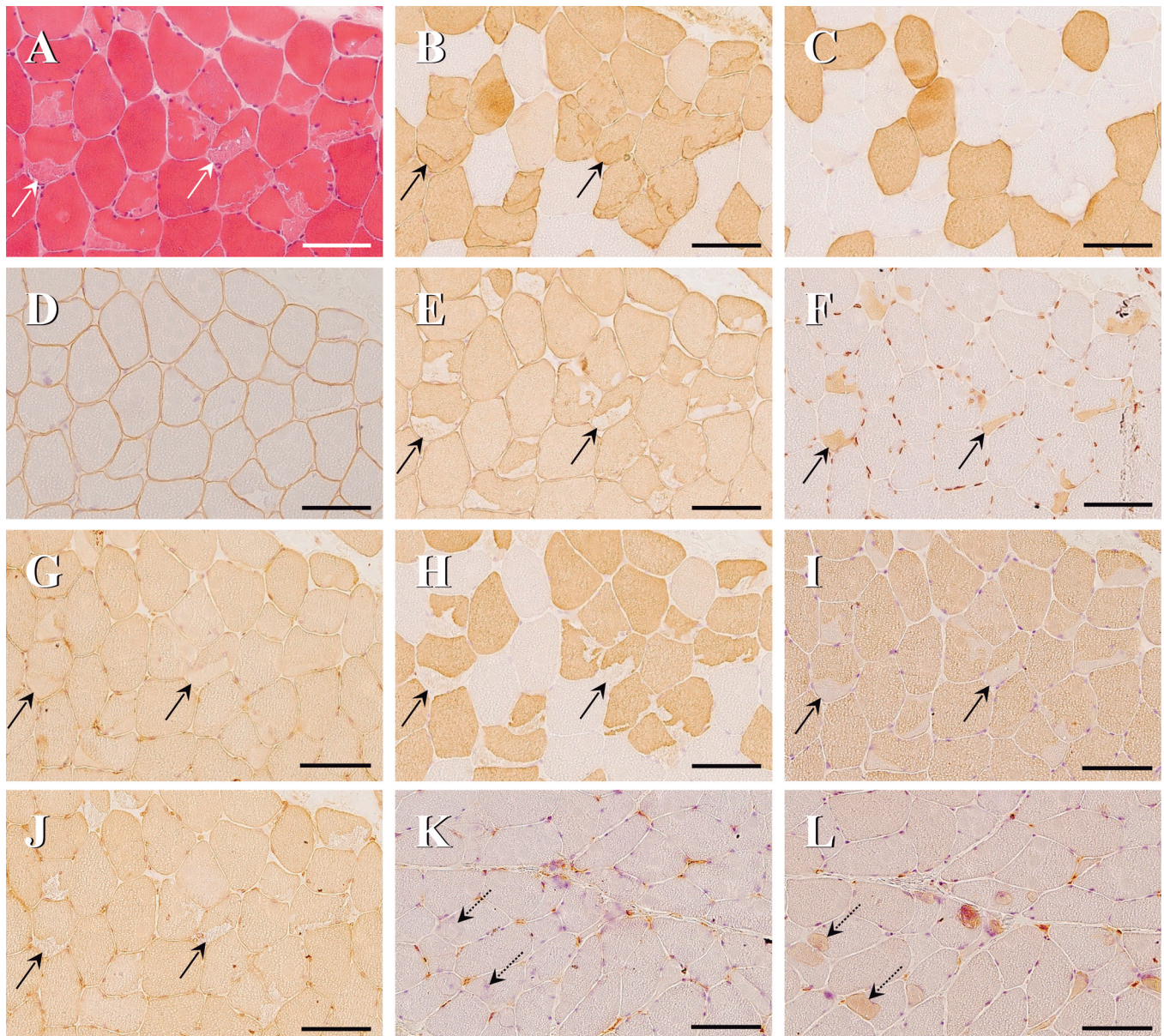


Fig. 5. Immunohistochemical features of large TAs **A-J**. Serial sections from the first biopsy (M. quadriceps) of patient 1017 (Table 1). **K, L**. Serial sections from the same biopsy of patient 1017. **A**. HE: large TAs are mainly located underneath the sarcolemma. **B**. SERCA-1: TAs show a stronger immunoreactivity than the SR of type 2 fibers. **C**. SERCA-2: no staining of the TAs. **D**. Dystrophin-3: TAs are not stained, while the sarcolemmal immunoreaction is normal. **E**. Desmin: no staining of the TAs. **F**. Emerin: positive immunoreactivity of the TAs and the nuclear membranes. **G**. Human fast skeletal myosin: TAs are unstained. **H**. MHC-f: TAs are unstained for the myosin heavy chain of type 2 fibers, while their sarcoplasm shows a positive immunoreactivity. **I**. Actin: the TAs are not stained for actin. **J**. Bigtau-404: no immunoreactivity of the TAs. **K**. Tubulin: no immunoreactivity of the TAs. **L**. Ubiquitin: positive immunoreactivity of the TAs for ubiquitin. Scale bars: 100 μ m.

family history of clinical symptoms and/or increased serum-CK was present. There was no known consanguinity. In patients 3.II.1 and 3.II.2 (Fig. 1) hyperornithinemia with gyrate atrophy of the choroids and retina was diagnosed. TAs of skeletal muscle fibers have been found earlier in this disease (Sipilä et al., 1979). In our study group with large TAs, we did not find another associated disorder or possible cause that could explain the presence of TAs in muscle fibers. In contrast, in the group with small TAs (77 biopsies), we noticed another associated disease in 50 cases (65%; summarized in Table 3) and a family history in only 4 patients (5%). The presence of small TAs in the muscle biopsy in our cohort is most frequently associated with mitochondrial disorder (14 patients or 18%), polyneuropathy or toxic drug-induced myopathy (Table 3). In the group of patients with small TAs associated with mitochondrial disorder, a genetic defect was identified in one patient (4977 bp deletion mtDNA). In two other patients, mutations responsible for MERRF, MELAS and NARP were excluded. In the remaining patients, molecular genetic analyses were not performed or the data were not available. The diagnosis of mitochondrial disorder was then based upon clinical features and muscle biopsy findings. We found no correlation between the ultrastructural data and clinical

presentations in either sub-group, or between the morphological features of TAs and the associated disorder in the sub-group with small TAs.

Discussion

We studied the histopathological and clinical spectrum in a large cohort of patients with TAs in their muscle biopsy. Based on the size of TAs, we defined two sub-groups: (1) myopathies with large TAs, characterized by the presence of TAs in type 2 fibers and sometimes also in type 1 fibers, the absence of another associated disorder, and a family history in half of the cases, and (2) myopathies with small TAs, presenting TAs exclusively in type 2 fibers, the presence of another associated disease in many patients and mostly no family history. No significant differences in clinical presentation, in age at onset or in disease duration at the time of biopsy were found between the two groups. We also observed a large variability in ultrastructural changes.

In our study, TAs occurred in 0.5% of the muscle biopsies from our archive (15,412 biopsies). Biopsies with small TAs exclusively visible by means of EM are included in this number. According to published data, the prevalence of TAs in muscle biopsies ranges from 0.2 to 1.6%: TAs were found in 1.6% (n=1,500, Engel et al., 1970), 0.6% (n=3,000, Niakan et al., 1985), 1.0% (n=1,500, Rosenberg et al., 1985), 0.2% (n=7,000, Oh et al., 2006) and 0.24% (n=7,441, Ghosh et al., 2010) of all included muscle biopsies.

We used the size of the TAs, measured by the surface area of the largest TA in transverse sections, as a criterion to further define and characterize these myopathies. We chose an arbitrary cut-off value of 125 μm^2 . In 0.19% of all biopsies, we found large TAs of at least 125 μm^2 in surface area. This sub-group might correspond to the so-called "primary TA myopathies (TAM)" (Dobkin and Verity, 1978; Rohkamm et al.,

Table 2. Symptoms in patients with myopathies with large and small TAs.

Symptoms	Large TAs	Small TAs	All
Limb weakness	16 (55%)	39 (51%)	52%
Myalgia	12 (41%)	34 (44%)	43%
Fatigability	8 (28%)	25 (32%)	31%
Muscle cramps	7 (24%)	13 (17%)	19%
Muscle stiffness	4 (14%)	8 (10%)	11%

Table 3. Associated diseases and conditions in cases with small tubular aggregates.

Associated disease	Number of biopsies (n = 76)	References
Mitochondrial disorder	14 (18 %)	Garrard et al., 2002; Novotová et al., 2002
Polyneuropathy	9 (12 %)	Fardeau et al., 1979; Niakan et al., 1985
Toxic / Drug-induced myopathy	5 (7 %)	Engel et al., 1970; Fardeau et al., 1979
Familial malignant hyperthermia	4 (5 %)	Gullotta and Helpap, 1975; Reske-Nielsen et al., 1975
Myotonic myopathy	4 (5 %)	Schröder and Becker, 1972
Alcohol-induced myopathy	4 (5 %)	Gullotta and Helpap, 1975; del Villar Negro et al., 1982
Hypokalemic periodic paralyses	2 (3 %)	Engel et al., 1970; Jurkat-Rott et al., 2000
Glycogenosis	2 (3 %)	Vissing et al., 1999; Oh et al., 2006
CADASIL	1 (1 %)	Schröder et al., 2005
Diabetic amyotrophy	1 (1 %)	Chokroverty et al., 1977
(Paraneoplastic) dermatomyositis	1 (1 %)	Rosenberg et al., 1985
Polymyositis	1 (1 %)	Grunnet et al., 1988
(Other) metabolic myopathy	1 (1 %)	-
M. Fabry	1 (1 %)	-
Without obvious association	26 (34 %)	-

1983; Pierobon-Bormioli, 1985; Martin et al., 1997; Müller et al., 2001; Vielhaber et al., 2001; Jacques et al., 2002; Shahrizaila et al., 2004; Ghosh et al., 2010), since many of these cases are familial and there is no other associated disease that might explain the morphological findings, and TAs are a prominent feature in the biopsy occurring in both type 1 and type 2 fibers. The sub-group showing TAs with a surface area smaller than $125 \mu\text{m}^2$ might have developed TAs as a “secondary and non-specific phenomenon” (Chevessier et al., 2005) that is associated with another disease that is non-familial and is restricted to type 2 fibers (Engel et al., 1970; Dubowitz and Sewry, 2007). We also considered other possible morphological criteria for primary TAM, such as the frequency of muscle fibers containing TAs. However, the frequency varied largely from 1- 90%, and no correlation between the maximal surface area and the frequency of TAs was found. Possible bias and limitations in using these morphological criteria are muscle selection for biopsy, and a sometimes unequal distribution of TAs within the same biopsy (Dubowitz and Sewry, 2007).

The presence of TAs in inbred mice is restricted to males (Agbulut et al., 2000; Chevessier et al., 2005). In humans, a sex-dependent prevalence of TAs has also been observed (Engel et al., 1970; Niakan et al., 1985; Rosenberg et al., 1985; Müller et al., 2001; Vielhaber et al., 2001; Oh et al., 2006; Ghosh et al., 2010). In our study, 90% of the index patients in the group with large TAs and 71% of the patients in the group with small TAs were male, corresponding to previous observations. TAs are usually seen in type 2 fibers (Engel et al., 1970; Dubowitz and Sewry, 2007). However, they can also occur in type 1 fibers in primary TAM (Dobkin and Verity, 1978; Rohkamm et al., 1983; Pierobon-Bormioli, 1985; Martin et al., 1997; Jacques et al., 2002; Shahrizaila et al., 2004; Ghosh et al., 2010). In our group with large TAs, we found some type 1 fibers containing TAs, but in the other biopsies there was predominance of TAs in type 2 fibers. A possible explanation is the “abundance of SR” in type 2 fibers compared to type 1 fibers (Shahrizaila et al., 2004). Furthermore, specific properties of metabolism, including differences in mitochondrial occurrence (Ogata and Yamasaki, 1997; Novotová et al., 2002), calcium homeostasis and protein expression might contribute to the TA formation in type 2 fibers.

At the EM level, a large variability in ultrastructural features was found in the group with large TAs. We found type I and type II tubules and the combination of these types in the majority of biopsies (50%, n=30). Type III tubules were most frequently found in combination with type I and type II tubules (30%), or rarely only in combination with type I tubules (7%). In contrast, type III tubules were never seen as a sole feature, or in combination with type II tubules. Interestingly, in one family (family 2), TFTAs with up to 38 tubules were observed. Assuming gradual *in vivo* TA formation (Engel et al., 1970) type III tubules could

represent a late stage of this process. The hypothesis of gradual composition is supported by biopsies of the extensor digitorum longus muscle (EDL) of mice of different ages (Boncompagni et al., 2012). In contrast, Schiaffino et al. (Schiaffino et al., 1977) showed the *in vitro* formation of TAs within hours induced by anoxia in the EDL of rats and they concluded that a rearrangement and/or aggregation of existing SR structures is more likely than an active neof ormation of TAs.

A comprehensive classification of tubular and cisternal systems of the muscle fiber was suggested by Schröder and Becker in 1972 (Schröder and Becker, 1972). They described tubules of 50 to 80 nm in diameter containing inner tubules (type Ib) and tubules of 40 to 50 nm in diameter without inner tubules (type Ic). These types have later been correlated with the type I and II tubules of Cameron and coworkers (Cameron et al., 1992; Müller et al., 2001). Schröder and Becker also characterized regularly and irregularly PTC of 80 to 90nm in diameter (type Ia) and giant tubules (type IIB; Schröder and Becker, 1972). We found FTTA and PTC each in about 20% of the biopsies with large TAs. VMC (13%) and TFTA (7%) were seen less frequently. Giant tubules were not present in the biopsies included in the present study.

The formation of TAs from the SR is supported by many studies (Schröder and Adams, 1968; Engel et al., 1970; Salviati et al., 1985; Martin et al., 1997; Chevessier et al., 2005). Furthermore, some data support the presence of mitochondria in TAs (Lewis et al., 1971; Rosenberg et al., 1985; Meijer, 1988; Vielhaber et al., 2001; Novotová et al., 2002). Activity of mitochondrial enzymes has been noted. Rosenberg et al. found TAs staining “reddish-brown” for SDH (Rosenberg et al., 1985). COX-positive TAs were described by Lewis et al. (Lewis et al., 1971). Meijer (1988) performed extensive histochemical adsorption studies on muscle sections containing TAs. High affinities of the TAs for NADH-TR and COX could be recognized. However, other mitochondrial enzymes showed a low affinity for TAs or had no affinity, such as SDH. Furthermore, the presence of the bc1-protein, which is an integral part of the inner mitochondrial membrane, could be shown in TAs by immunogold-labeling; the study was conducted in mice with invalidated cytosolic and mitochondrial creatin kinase (Novotová et al., 2002). In our study, in the three biopsies of family 1 and in 6 additional unrelated individuals, the TAs stained strongly with SDH, whereas the other cases were negative. For the mixed results on mitochondrial participation in TA formation, different possible explanations exist. Firstly, there may be different mechanisms of TA emergence, with some TAs partly assembling from mitochondria (Meijer, 1988). Secondly, a diffusion of mitochondrial enzymes into TAs might take place, particularly of NADH-TR because of its high affinity to TAs (Meijer, 1988). In contrast, COX-positive TAs are seen very rarely (Lewis et al., 1971), in spite of their high COX-affinity (Meijer, 1988). Thirdly,

an unspecific reaction could take place and might be misinterpreted as mitochondrial enzymatic activity. Such an unspecific reaction has for example been shown for enzyme assays based on tetranitroblue tetrazolium (TNBT), particularly in AMPD and NADH-TR assays (Fuchs and Wohlrab, 1967; Higuchi et al., 1987). However, we have excluded the possibility of an unspecific reaction by confirming our results using an anti-SDH-antibody. Further studies will be necessary to clarify this issue.

Several hypotheses about the function of TAs in muscle fibers have been suggested, such as an adaptive response of the muscle fibers to increased intracellular calcium flux or a functional link with mitochondrial dysfunction and abnormal intracellular energy metabolism (Salviati et al., 1985; Steeghs et al., 1997; Vielhaber et al., 2001). Heat-shock proteins have also been suggested to play a role in the formation of TAs (Martin et al., 1991). We tested the TAs for alpha-B-crystallin as a heat-shock protein, but no immunoreactivity was revealed. Furthermore, we found expression of proteins that are not directly related to the SR, such as dysferlin and emerin, as has been previously reported in TAs (Ikezoe et al., 2003; Vita et al., 2003; Manta et al., 2004). Ikezoe et al. suggested that dysferlin could be expressed in response to disturbed sarcoplasmic Ca^{2+} -homeostasis (Ikezoe et al., 2003). Manta and coworkers (Manta et al., 2004) described emerin-positive TAs and proposed a possible mutation in emerin leading to its mislocalization into the SR membrane instead of the inner nuclear membrane. We also found ubiquitin- and tau positive TAs, as previously shown by Luan et al. (2009). Ubiquitin in TAs probably plays a role in labeling accumulated proteins for degradation (Luan et al., 2009). The accumulation of tau shows a possible link to neurodegenerative diseases, as has been recently suggested by Schiaffino (2012). He proposed that TAs are a special type of protein aggregates comparable to protein aggregates in neurodegenerative diseases. Thus, the pathophysiology of TAs seems to be diverse, and further insights might come from future studies in genetically confirmed families with tubular aggregate myopathy.

As a practical clinico-pathological guideline, one should think of myopathy with TAs as a possible diagnosis in a patient presenting with limb muscle weakness, myalgia, fatigability, muscle cramps and/or stiffness. A muscle biopsy and AMPD and/or NADH-TR reaction should be performed, which would reveal positively stained TAs. The AMPD-reaction stains TAs, whereas the NADH-TR reaction stains TAs and mitochondria. In addition, electron microscopic studies are recommended. Furthermore, it would be interesting to measure the size of the TAs in transverse sections of muscle fibers, using a cut-off value of $125 \mu m^2$ to distinguish between large TAs ($\geq 125 \mu m^2$) and small TAs ($< 125 \mu m^2$). The distinction between myopathies with large TAs and myopathies with small TAs is important, because the myopathies with large TAs most

probably have a primary genetic cause, indicated by their familial occurrence without other associated disorders. This group of myopathies with large TAs will in further studies be used for the identification of causative genes using novel genetic techniques. Finding the underlying genes in this myopathy will have clinical relevance and might lead to a better understanding of underlying pathomechanisms. In myopathies with small TAs, another associated disease is present in most cases, and a family history is unlikely. The most frequent associated disorder in muscle biopsies showing small TAs that should be looked for is a mitochondrial disease. To date, no curative treatment is available for myopathies with TAs.

Acknowledgements. We would like to thank the technical and administrative personnel of the Institute of Neuropathology and the Department of Neurology at the RWTH Aachen University, Aachen (Germany). We are grateful to the referring colleagues for sending the biopsies for further studies. We thank Dr. G. Brook, Institute of Neuropathology, RWTH Aachen University, for correcting and editing our manuscript.

References

- Agbulut O., Destombes J., Thiesson D. and Butler-Browne G. (2000). Age-related appearance of tubular aggregates in the skeletal muscle of almost all male inbred mice. *Histochem. Cell Biol.* 114, 477-481.
- Alonso-Losada G., Cimas I., Pego R., Torre P.L., Teixeira S. and Navarro C. (1998). Isolated progressive muscle weakness with tubular aggregates. *Clin. Neuropathol.* 17, 50-54.
- Belaya K., Finlayson S., Slater C.R., Cossins J., Liu W.W., Maxwell S., McGowan S.J., Maslau S., Twigg S.R.F., Walls T.J., Pascual Pascual S.I., Palace J. and Beeson D. (2012). Mutations in DPAGT1 cause a limb-girdle congenital myasthenic syndrome with tubular aggregates. *Am. J. Hum. Genet.* 91, 193-201.
- Boncompagni S., Protasi F. and Franzini-Armstrong C. (2012). Sequential stages in the age-dependent gradual formation and accumulation of tubular aggregates in fast twitch muscle fibers: SERCA and calsequestrin involvement. *Age (Dordr)* 34, 27-41.
- Cameron C.H., Allen I.V., Patterson V. and Avaria M.A. (1992). Dominantly inherited tubular aggregate myopathy. *J. Pathol.* 168, 397-403.
- Chevessier F., Bauché-Godard S., Leroy J.-P., Koenig J., Paturneau-Jouas M., Eymard B., Hantaï D. and Verdière-Sahuqué M. (2005). The origin of tubular aggregates in human myopathies. *J. Pathol.* 207, 313-323.
- Chokroverty S., Reyes M.G., Rubino F.A. and Tonaki H. (1977). The syndrome of diabetic amyotrophy. *Ann. Neurol.* 2, 181-194.
- del Villar Negro A., Merino Angulo J., River Pomar J.M. and Aguirre Errasti C. (1982). Tubular aggregates in skeletal muscle of chronic alcoholic patients. *Acta Neuropathol.* 56, 250-254.
- Dobkin B.H. and Verity M.A. (1978). Familial neuromuscular disease with type 1 fiber hypoplasia, tubular aggregates, cardiomyopathy, and myasthenic features. *Neurology* 28, 1135-1140.
- Dubowitz V. and Sewry C.A. (2007). *Muscle biopsy. A practical approach.* 3rd ed. London: Saunders Elsevier.
- Engel W.K. (1964). Mitochondrial aggregates in muscle disease. *J.*

Pathological spectrum in TA myopathies

- Histochem. Cytochem. 12, 46-48.
- Engel W.K., Bishop D.W. and Cunningham G.G. (1970). Tubular aggregates in type II muscle fibers: ultrastructural and histochemical correlation. *J. Ultrastruct. Res.* 31, 507-525.
- Fardeau M., Tomé F.M. and Simon P. (1979). Muscle and nerve changes induced by perhexiline maleate in man and mice. *Muscle Nerve* 2, 24-36.
- Fuchs U. and Wohlrab F. (1967). Non-enzymatically conditioned TNBT-formazan deposits in tissue structures. *Experientia* 23, 390-392. (In German)
- Furui E., Fukushima K., Sakashita T., Sakato S., Matsubara S. and Takamori M. (1997). Familial limb-girdle myasthenia with tubular aggregates. *Muscle Nerve* 20, 599-603.
- Garrard P., Blake J., Stinton V., Hanna M.G., Reilly M.M., Holton J.L., Landon D.N. and Honan W.P. (2002). Distal myopathy with tubular aggregates: a new phenotype associated with multiple deletions in mitochondrial DNA? *J. Neurol. Neurosurg. Psychiatry* 73, 207-208.
- Ghosh A., Narayanappa G., Taly A.B., Chickbasavaiya Y.T., Mahadevan A., Vani S., Atchayaram N., Mohapatra I. and Susarala K.S. (2010). Tubular aggregate myopathy: a phenotypic spectrum and morphological study. *Neurol. India* 58, 747-751.
- Grunnet M.L., Abeles M. and Hofbauer H. (1988). Polymyositis with tubular aggregates. *J. Rheumatol.* 15, 1288-1290.
- Guergueltcheva V., Müller J.S., Dusl M., Senderek J., Oldfors A., Lindbergh C., Maxwell S., Colomer J., Mallebrera C.J., Nascimento A., Vilchez J.J., Muelas N., Kirschner J., Nafissi S., Kariminejad A., Nilipour Y., Bozorgmehr B., Najmabadi H., Rodolico C., Sieb J.P., Schlotter B., Schoser B., Herrmann R., Voit T., Steinlein O.K., Najafi A., Urtizberea A., Soler D.M., Muntoni F., Hanna M.G., Chaouch A., Straub V., Bushby K., Palace J., Beeson D., Abicht A. and Lochmüller H. (2011). Congenital myasthenic syndrome with tubular aggregates caused by GFPT1 mutations. *J. Neurol.* 259, 838-850.
- Gullotta F. and Helpap B. (1975). Histological, histochemical, and ultrastructural findings in malignant hyperthermia (author's transl). *Virchows Arch. A* 367, 181-194. (In German)
- Higuchi I., Ishiura S., Nonaka I. and Sugita H. (1987). Immunohistochemical localization of AMP deaminase in rimmed vacuoles in human skeletal muscle. *Muscle Nerve* 10, 790-800.
- Huh S.-Y., Kim H.-S., Jang H.-J., Park Y.-E. and Kim D.-S. (2012). Limb-girdle myasthenia with tubular aggregates associated with novel GFPT1 mutations. *Muscle Nerve* 46, 600-604.
- Ikezoe K., Furuya H., Ohyagi Y., Osoegawa M., Nishino I., Nonaka I. and Kira J.-I. (2003). Dysferlin expression in tubular aggregates: their possible relationship to endoplasmic reticulum stress. *Acta Neuropathol.* 105, 603-609.
- Jacques T.S., Holton J., Watts P.M., Wills A.J., Smith S.E. and Hanna M.G. (2002). Tubular aggregate myopathy with abnormal pupils and skeletal deformities. *J. Neurol. Neurosurg. Psychiatry* 73, 324-326.
- Jurkat-Rott K., Mitrovic N., Hang C., Kouzmekine A., Iazzo P., Herzog J., Lerche H., Nicole S., Vale-Santos J., Chauveau D., Fontaine B. and Lehmann-Horn F. (2000). Voltage-sensor sodium channel mutations cause hypokalemic periodic paralysis type 2 by enhanced inactivation and reduced current. *Proc. Natl. Acad. Sci. USA* 97, 9549-9554.
- Lewis P.D., Pallis C. and Pearse A.G. (1971). "Myopathy" with tubular aggregates. *J. Neurol. Sci.* 13, 381-388.
- Luan X., Chen B., Liu Y., Zheng R., Zhang W. and Yuan Y. (2009). Tubular aggregates in paralysis periodica paramyotonia with T704M mutation of SCN4A. *Neuropathology* 29, 579-584.
- Manta P., Terzis G., Papadimitriou C., Kontou C. and Vassilopoulos D. (2004). Emerin expression in tubular aggregates. *Acta Neuropathol.* 107, 546-552.
- Martin J.E., Mather K., Swash M. and Gray A.B. (1991). Expression of heat shock protein epitopes in tubular aggregates. *Muscle Nerve* 14, 219-225.
- Martin J.J., Ceuterick C. and Goethem G.V. (1997). On a dominantly inherited myopathy with tubular aggregates. *Neuromuscul. Disord.* 7, 512-520.
- Meijer A.E. (1988). Histochemical features of tubular aggregates in diseased human skeletal muscle fibres. *J. Neurol. Sci.* 86, 73-82.
- Müller H.D., Vielhaber S., Brunn A. and Schröder J.M. (2001). Dominantly inherited myopathy with novel tubular aggregates containing 1-21 tubulofilamentous structures. *Acta Neuropathol.* 102, 27-35.
- Niakan E., Harati Y. and Danon M.J. (1985). Tubular aggregates: their association with myalgia. *J. Neurol. Neurosurg. Psychiatry* 48, 882-886.
- Novotová M., Zahradník I., Brochier G., Pavlovicová M., Bigard X. and Ventura-Clapier R. (2002). Joint participation of mitochondria and sarcoplasmic reticulum in the formation of tubular aggregates in gastrocnemius muscle of CK-/- mice. *Eur. J. Cell Biol.* 81, 101-106.
- Ogata T. and Yamasaki Y. (1997). Ultra-high-resolution scanning electron microscopy of mitochondria and sarcoplasmic reticulum arrangement in human red, white, and intermediate muscle fibers. *Anat. Rec.* 248, 214-223.
- Oh S.J., Park K.-S., Ryan H.F., Danon M.J., Lu J., Naini A.B. and DiMauro S. (2006). Exercise-induced cramp, myoglobinuria, and tubular aggregates in phosphoglycerate mutase deficiency. *Muscle Nerve* 34, 572-576.
- Pavlovicová M., Novotová M. and Zahradník I. (2003). Structure and composition of tubular aggregates of skeletal muscle fibres. *Gen. Physiol. Biophys.* 22, 425-440.
- Pierobon-Bormioli S., Armani M., Ringel S.P., Angelini C., Vergani L., Betto R. and Salviati G. (1985). Familial neuromuscular disease with tubular aggregates. *Muscle Nerve* 8, 291-298.
- Reske-Nielsen E., Haase J. and Kelstrup J. (1975). Malignant hyperthermia in a family. The ultrastructure of muscle biopsies of healthy members. *Acta. Pathol. Microbiol. Scand. A* 83, 651-660.
- Rohkamm R., Boxler K., Ricker K. and Jerusalem F. (1983). A dominantly inherited myopathy with excessive tubular aggregates. *Neurology* 33, 331-336.
- Rosenberg N.L., Neville H.E. and Ringel S.P. (1985). Tubular aggregates. Their association with neuromuscular diseases, including the syndrome of myalgias/cramps. *Arch. Neurol.* 42, 973-976.
- Salviati G., Pierobon-Bormioli S., Betto R., Damiani E., Angelini C., Ringel S.P., Salvatori S. and Margreth A. (1985). Tubular aggregates: sarcoplasmic reticulum origin, calcium storage ability, and functional implications. *Muscle Nerve* 8, 299-306.
- Schiaffino S. (2012). Tubular aggregates in skeletal muscle: Just a special type of protein aggregates? *Neuromuscul. Disord.* 22, 199-207.
- Schiaffino S., Severin E., Cantini M. and Sartore S. (1977). Tubular aggregates induced by anoxia in isolated rat skeletal muscle. *Lab. Invest.* 37, 223-228.
- Schröder J.M. and Adams R.D. (1968). The ultrastructural morphology of the muscle fiber in myotonic dystrophy. *Acta Neuropathol.* 10, 218-241.

- Schröder J.M. and Becker P.E. (1972). Alterations of the T-system and the sarcoplasmic reticulum in myotonia, paramyotonia and adynamia. *Virchows Arch. A* 357, 319-344.
- Schröder J.M., Züchner S., Dichgans M., Nagy Z. and Molnar M.J. (2005). Peripheral nerve and skeletal muscle involvement in CADASIL. *Acta Neuropathol.* 110, 587-599.
- Senderek J., Müller J.S., Dusl M., Strom T.M., Guergueltcheva V., Diepolder I., Laval S.H., Maxwell S., Cossins J., Krause S., Muelas N., Vilchez J.J., Colomer J., Mallebrera C.J., Nascimento A., Nafissi S., Kariminejad A., Nilipour Y., Bozorgmehr B., Najmabadi H., Rodolico C., Sieb J.P., Steinlein O.K., Schlotter B., Schoser B., Kirschner J., Herrmann R., Voit T., Oldfors A., Lindbergh C., Urtizberea A., von der Hagen M., Hübner A., Palace J., Bushby K., Straub V., Beeson D., Abicht A. and Lochmüller H. (2011). Hexosamine biosynthetic pathway mutations cause neuromuscular transmission defect. *Am. J. Hum. Genet.* 88, 162-172.
- Shahrizaila N., Lowe J. and Wills A. (2004). Familial myopathy with tubular aggregates associated with abnormal pupils. *Neurology* 63, 1111-1113.
- Shy G., Wanko T., Rowley P.T. and Engel A.G. (1961). Studies in familial periodic paralysis. *Exp. Neurol.* 3, 53-121.
- Sieb J.P., Tolksdorf K., Dengler R. and Jerusalem F. (1996). An autosomal-recessive congenital myasthenic syndrome with tubular aggregates in a Libyan family. *Neuromuscul. Disord.* 6, 115-119.
- Sipilä I., Simell O., Rapola J., Sainio K. and Tuuteri L. (1979). Gyrate atrophy of the choroid and retina with hyperornithinemia: tubular aggregates and type 2 fiber atrophy in muscle. *Neurology* 29, 996-1005.
- Steeghs K., Benders A., Oerlemans F., de Haan A., Heerschap A., Ruitenbeek W., Jost C., van Deursen J., Perryman B., Pette D., Brückwilder M., Koudijs J., Jap P., Veerkamp J. and Wieringa B. (1997). Altered Ca²⁺ responses in muscles with combined mitochondrial and cytosolic creatine kinase deficiencies. *Cell* 89, 93-103.
- Vielhaber S., Schröder R., Winkler K., Weis S., Sailer M., Feistner H., Heinze H.J., Schröder J.M. and Kunz W.S. (2001). Defective mitochondrial oxidative phosphorylation in myopathies with tubular aggregates originating from sarcoplasmic reticulum. *J. Neuropathol. Exp. Neurol.* 60, 1032-1040.
- Vissing J., Schmalbruch H., Haller R.G. and Clausen T. (1999). Muscle phosphoglycerate mutase deficiency with tubular aggregates: effect of dantrolene. *Ann. Neurol.* 46, 274-277.
- Vita G., Monici M.C., Owaribe K. and Messina C. (2003). Expression of plectin in muscle fibers with cytoarchitectural abnormalities. *Neuromuscul. Disord.* 13, 485-492.

Accepted March 6, 2013