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SYMPOSIUM: Recent Advances in Hereditary Neuromuscular Diseases of Childhood

Congenital Myopathies

Antje Bornemann[1] and Hans H. Goebel[2]

[1] Institute of Brain Research, Eberhard-Karls University, Tübingen Germany
[2] Department of Neuropathology, Johannes Gutenberg University, Mainz, Germany

Most congenital myopathies have been defined on account of the morphological findings in enzyme histochemical preparations. In effect, the diagnosis of this group of diseases continues to be made on the histological pattern of muscle biopsies. However, progress has been made in elucidating the molecular genetic background of several of the congenital myopathies. In this updated review we address those congenital myopathies for which gene defects and mutant proteins have been found (central core disease, nemaline myopathies, desminopathy, actinopathy, certain vacuolar myopathies, and myotubular myopathy) and the other disease with central nuclei (centronuclear myopathy).

Introduction

Congenital myopathies (CM) are considered a separate category of muscle disorders on account of their striking and characteristic histopathological patterns. Certain conditions have only been described once or twice, often many years ago, such as sarcotubular myopathy, fingerprint body myopathy, reducing body myopathy, trilaminar fibre myopathy, cap disease, or zebra body myopathy. New cases with hitherto undescribed morphological abnormalities continue to be observed such as "myopathy with hexagonally cross-linked tubular arrays" (8). Extensive reviews have been provided (24, 33, 34) and, recently, in conjunction with a European Neuromuscular Centre (ENMC) workshop (30). In particular, the increasing numbers of workshops and consortia procured by the ENMC on different groups of congenital myopathies have contributed to a renewed interest as well as new data in many CM.

The morphology-based established classification of some 40 CM (33) has met with reconsideration, since new genes have been identified recently in several CM. A case in point is nemaline myopathy. Several clinical types of nemaline myopathy have now been identified to consist of several separate genetic disorders, so far termed nemaline myopathies or NEM1-4, with additional affected genes to be expected. As congenital myopathies have been defined by morphological abnormalities, often inclusion bodies, several such inclusion bodies, such as cytoplasmic, spheroid, and sarcoplasmic bodies, or granulofilamentous material have been found to contain proteins in excess, some of them even mutant, *e.g.* desmin in desminopathies, and α-B crystallin in α-B crystallinopathy, or actin in actinopathy, suggesting a separate subcategory among the CM, one of "protein surplus myopathies." Immunohistochemical identification of such proteins accumulating within pathological structures of certain CM have presented candidate proteins for respective mutation analyses in their genes, the desmin, α-B crystallin, and actin genes.

In this chapter we discuss those CM of which new immunohistochemical and molecular data have been recorded (Table 1).

Desmin-related myopathies (DRM)

Among the congenital myopathies marked by inclusion bodies, certain types, i.e. cytoplasmic bodies and sarcoplasmic bodies have been found to be associated with the deposition of the muscle fibre-specific intermediate filament desmin (21,66). With the major introduction of immunohistochemistry to myopathology, other conditions and myopathological features became associated with the accumulation of desmin: granulofilamentous material (23, 37, 70) Mallory body-like inclusions (26, 27), and spheroid bodies (32). Thus, currently, increase in desmin within muscle fibres in congenital myopathies may be found in conjunction with inclusions such as: (a) sarcoplasmic bodies, cytoplasmic bodies, and spheroid bodies (Figure 1a, d) (32); (b) granulofilamentous material (Figure 1b, c) which is more diffusely (though with subsarcolemmal preponderance) spread across the muscle fibre area; (c) immature fibres, not only regenerating ones, but also those present in

Corresponding author:
Antje Bornemann, Institute of Brain Research, Eberhard-Karls University, Calwerstrasse 3, 72076 Tübingen Germany; Tel.: +49 7071 29 80162; Fax: +49 7071 29 4846, E-mail: antje.bornemann@med.uni-tuebingen.de

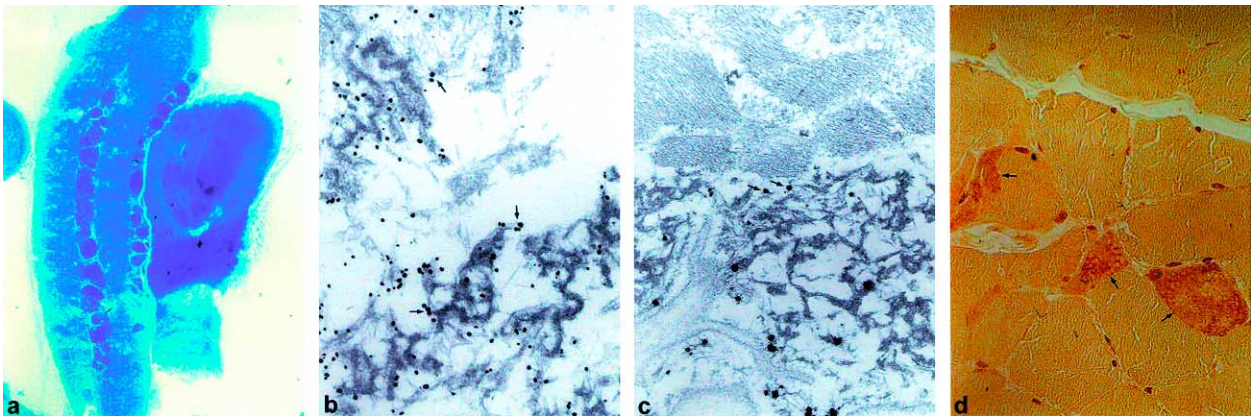

Figure 1. Different aspects of desmin-related myopathy. (**a**) Spheroid bodies are lined up in the centre of a longitudinally cut muscle fibre, *spheroid body myopathy*, modified trichrome stain. Magnification: ×375. (**b**) Desmin is marked by the immunogold technique with silver enhancement on numerous filaments (arrows) and largely spares electron-dense granular material, *granulofilamentous myopathy*. Magnification: ×46,500. (**c**) Also, dystrophin is present in filaments (arrows) and outside of the electron-dense granular material, *granulofilamentous myopathy*, immunogold technique with silver enhancement. Magnification ×31,500. (**d**) Muscle fibres are completely or partially labelled (arrows) with an antibody against ubiquitin, *spheroid body myopathy*. Magnification: ×400.

myotubular myopathy (73) and centronuclear myopathy (59), in these latter congenital myopathies appearing in a diffuse fashion within muscle fibres.

DRM encompass only those which are marked by not only a multifocal excess or surplus of desmin (Figure 1b), the inclusion-body and granulofilamentous types, but also by accumulation of other proteins within these lesions. This had been shown in numerous publications and recently resulted in using DRM synonymously with the rather generic term myofibrillar myopathy (18, 62). The spectrum of accruing proteins in DRM is quite diverse, some seem to be regularly present such as dystrophin (Figure 1c), nestin, nebulin; others seem to be aberrant ones such as chaperone proteins ubiquitin (Figure 1d) and α-B crystallin, α1-antichymotrypsin, gelsolin and protein epitopes related to β-A4 amyloid usually encountered in aged and Alzheimer disease brains. Little is known of why and how these proteins accumulate and, in which way these accumulating proteins are abnormal, is likewise little understood.

Few pertinent data are available from the accrued desmin. Firstly, similar to phosphorylation of neurofilaments in Lewy bodies, hyperphosphorylation of the respective muscle fibre-related intermediate filament, desmin, has been shown (11, 70). An increase in acidic isoforms apparently contributes to deposition of soluble desmin (11), and explains why desmin filaments my not always be recognized (62), although desmin is immunohistochemically increased (18).

Secondly, recent studies (16, 39, 40, 60, 72, 77) demonstrated mutations in the desmin gene (Table 2) in several familial and sporadic DRM and even a primary

Congenital myopathy:	Gene locus:	Protein product:
Nemaline myopathy	NEM 1: 1q21-23	α-tropomyosin [TPM3]
	NEM 2: 2q21.2-22	nebulin [NEB]
	NEM 3: 1q42.1	sarcomeric actin [ACTA1]
	NEM 4: 9p13.2	β-tropomyosin [TPM2]
Central core disease [CCD]	19q13.1	Ryanodine receptor [RYR1]
α-B crystallinopathy	11q22	α-B crystallin [CRYAB]
Myotubular myopathy [MTMX]	X28q	Myotubularin
Desminopathy	2q35	Desmin [DES]
Desmin-related myopathies	2q21	*Unknown*
	10q	*Unknown*
	12	*Unknown*
Vacuolar myopathies		
Kalimo type (XMEA)	Xq28	*Unknown*
Muntoni type	Xq24	LAMP-2 (lysosome-associated Membrane protein B)

Table 1. Molecular Genetics in Congenital Myopathies.

cardiomyopathy (49), justifying the term desminopathies, in analogy to the terms dystrophinopathies and sarcoglycanopathies. Heterozygous mutations in the desmin gene apparently result in the presence of both, wild type and mutant desmins. Actually, two bands of desmin have, by immunoblot, been documented in a desmin-related myopathy of a mother and her daughter (51), although no mutational data were included. Mutant desmins, perhaps, form the primary core of desmin aggregates and, thereby, trap/bind and prevent other proteins from intracellular degradation including the assembly of hitherto unknown proteins together with desmin, such as gelsolin and β-A4 amyloid-related epitopes.

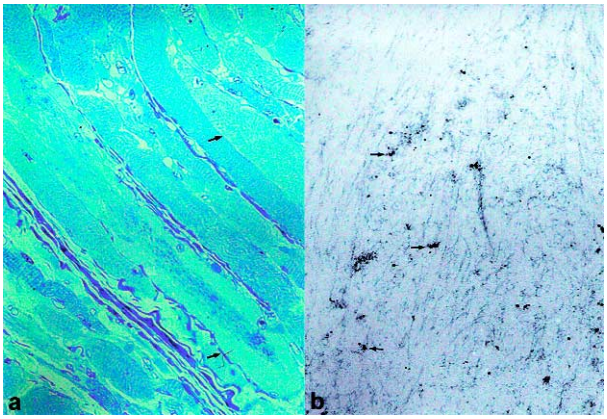

Figure 2. Actinopathy. (**a**) Large homogeneous light areas within muscle fibres (arrows), representing actin filaments, 1μm-thick plastic section, methylene blue. Magnification: ×350. (**b**) Numerous thin filaments are labelled with immunogold grains against actin (arrows). Magnification: ×31,950.

Type :	Location: exon/intron	Inheritance :	Mutant protein :	Reference:
Missense	Exon 5 G to C	AD: heterozygous	Ala337 Pro rod domain, 2B helix subdomain	(39)
Missense	2x exon 6 G to C A to T	AR: Compound heterozygous	Asn 393 Ile Ala 360 Pro Rod domain 2B helix subdomain	(39)
Deletion	Exon 3	AR: Homozygous	del. 7aa (R173 E179) rod domain 1B helix subdomain	(60)
Missense	Exon 8 C to G	AD: Heterozygous	Ile 451 Met carboxy tail domain	(49) cardio-myopathy only
Missense	Exon 8	AD: Heterozygous	Ile 451 Met carboxy tail domain	(16)
Missense	Exon 6 A to G	AD: Heterozygous	Asn 342 Asp rod domain 2B helix subdomain	(16)
Missense	Exon 6 C to T	AD: Heterozygous	Arg 406 Trp rod domain 2B helix subdomain	(16)
Missense	T to C	AD: Heterozygous	Leu 345 Pro rod domain 2B helix subdomain	(77)
Splicing defect	Intron 3 A to G	AD: Heterozygous	del. 32aa rod domain 1B helix subdomain	(16)
Splicing defect	Intron 2 G to A	AD: Heterozygous	del. exon 3	(67)

Table 2. Current mutations in the desmin gene.

A missense mutation in the α-B crystallin, a protein of the small heat shock protein family which also accumulates with desmin (29, 37, 86) had further suggested that perhaps other mutant proteins accumulate in those DRM, desmin mutations of which have been excluded (16). Linkage to chromosomes 2 (94), 10 (57), and 12 (92) in DRM support such an assumption although proteins associated with these mutated genes have not yet been identified.

Recognition of accumulation as well as mutation of desmin in DRM have further broadened the definition of congenital myopathies as early-onset neuromuscular conditions in that the majority of DRM are of late onset, often mimicking distal myopathies (35, 42). Furthermore, a number of patients not only suffers from skeletal myopathy, but also from cardiomyopathy, another expansion of the clinical spectrum of congenital myopathies.

DRM show, in common with hereditary inclusion body myopathies, a multitude of proteins accumulating within muscle fibres some of which, *e.g.* β-A4 amyloid-related ones as well as α1-antichymotrypsin, may occur in both groups of neuromuscular conditions (4, 18). However, mutant proteins have not yet been identified in hereditary inclusion body myopathies which are morphologically marked by accumulation of tubulofilaments in muscle fibres.

The characteristic ultrastructural component of desmin aggregates in DRM is granulofilamentous material, which is similarly encountered in other cell types of other, usually neurodegenerative disorders: Lewy bodies with neurofilaments in Parkinson disease and Rosenthal fibres with glial fibrillary acidic protein in Alexander disease. Mutant proteins have also been identified in these non-muscular lesions: mutant α-synuclein in Lewy bodies of familial juvenile Parkinson disease (28) and mutant glial fibrillary acidic protein in Alexander disease (58). As regards ultrastructural and certain immunohistochemical findings, DRM and desmin-accumulating myofibres, hence, resemble certain neurodegenerative disease such as Lewy body disease, Parkinson disease as well as Alexander disease. Similarly, Lewy bodies and Rosenthal fibres may also be encountered in other conditions such as aging and neoplasia, respectively. Accumulation of desmin may also occur without mutant desmin or mutant α-B crystallin present (16, 40). In this respect, muscle fibres and nerve cells apparently share such filamentous aggregates, perhaps, owing to their longevity and their incapability to rid themselves of surplus, non-usable endogenous proteins.

Actinopathies

The excess or surplus of proteins within muscle fibres in DRM renders this group of myopathies a member of the emerging protein surplus myopathies, another neuromuscular condition of which, actinopathy, is a

congenital myopathy of the nemaline type (31) marked by accumulation of mutant actin (Figure 2) (38, 65). Accordingly, such a congenital myopathy with accumulating mutant actin has been termed actinopathy. It has been found among the nemaline myopathies (65) but, apparently, also without any rod formation (5, 20, 31, 65).

Other protein surplus myopathies

A possible other protein surplus myopathy of the congenital type is hyaline body myopathy, marked by accumulation of circumscribed patches of finely granular material (Figure 3) which is rich in ATPase activity and myosin (3, 12, 55). Despite familial occurrence of this hyaline body myopathy (10, 55), no gene mutation or mutant protein has yet been identified.

Nemaline myopathies

Historical development. Contrary to common knowledge, that nemaline myopathy was described almost 40 years ago (13, 76) it has now been disclosed (74) that nemaline myopathy and nemaline/rod bodies were actually discovered five years earlier, in 1958, in paraffin sections of a biopsied muscle from a three-year-old boy at the Royal Alexandra Hospital of Sydney/Australia by the pathologist Douglas Reye (Reye syndrome), but had remained in oblivion. That this young boy's neuromuscular condition was indeed nemaline myopathy has also recently been confirmed by showing a missense mutation in the ACTA1 gene of this now 45-year-old patient (74) also conforming to a mild course among the several clinical types of nemaline myopathy. This mutational study has not been performed on archival tissues, but on the living patient's recently obtained DNA.

Largely by a concerted effort of the European Neuromuscular Centre (ENMC) and ENMC-inspired studies (31, 36, 38, 65, 89, 90), nemaline myopathy has undergone profound re-evaluation providing precise diagnostic criteria for several clinical subtypes (91) including reports on fetal nemaline myopathy, or fetal akinesia sequence (46), as well as associated cardiomyopathy (78) and vigorous molecular research.

Genes and mutations: A juvenile slowly progressive form of nemaline myopathy, inherited in an autosomal-dominant mode, was found to have a missene mutation in the α-tropomyosin gene (*TPM3*) located on chromosome 1q22-q23 (45). This was the first gene mutation in nemaline myopathy, *NEM1*. How the Met9Arg mis-

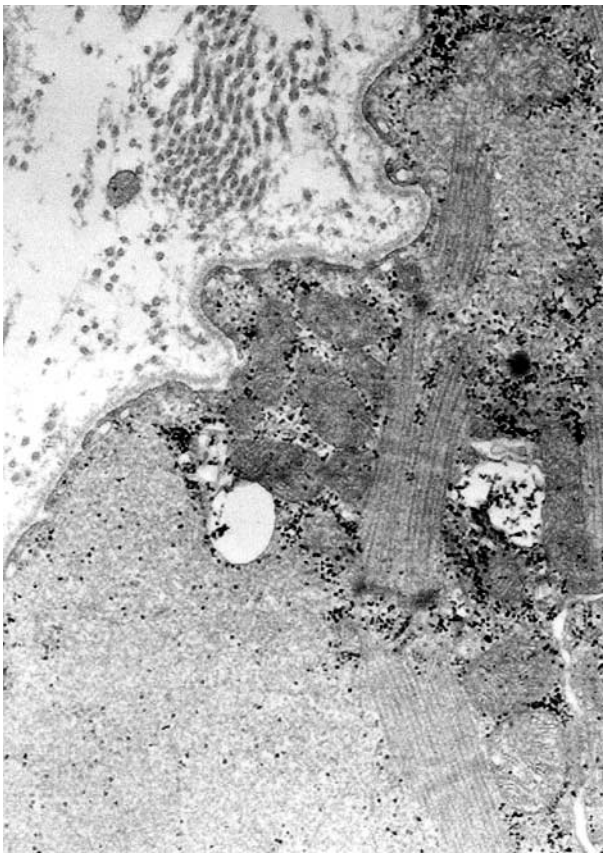

Figure 3. Hyaline body myopathy. Large subsarcolemmal area replete with finely granular material and sharply demarcated from adjacent sarcomeres, hyaline plaque. Magnification: ×10,000.

sense mutation affects the formation of rods — in this family only observed in the sarcoplasm — or how it causes weakness is unknown. Tropomyosin belongs to the family of thin filaments related to actin. The binding of mutant *TPM3* to actin is most likely disturbed, perhaps even resulting in stronger tropomyosin-actin binding and, thus, impairing regular Z-disk formation and favouring formation of rods (45). Another mutation, a nonsense mutation of the *TPM3* gene was found homozygously present in another patient with the severe infantile form of nemaline myopathy (80). As α-tropomyosin is more expressed in type-I muscle fibres, the rods occur in type-I muscle fibres only in respective biopsied muscle tissues (45, 80).

As α-tropomyosin forms a dimer with β-tropomyosin, it was not surprising to find, almost seven years later, a mutation in the β-tropomyosin gene (TPM2), β-tropomyosin being located on chromosome 9, in an apparent different genetic form of nemaline

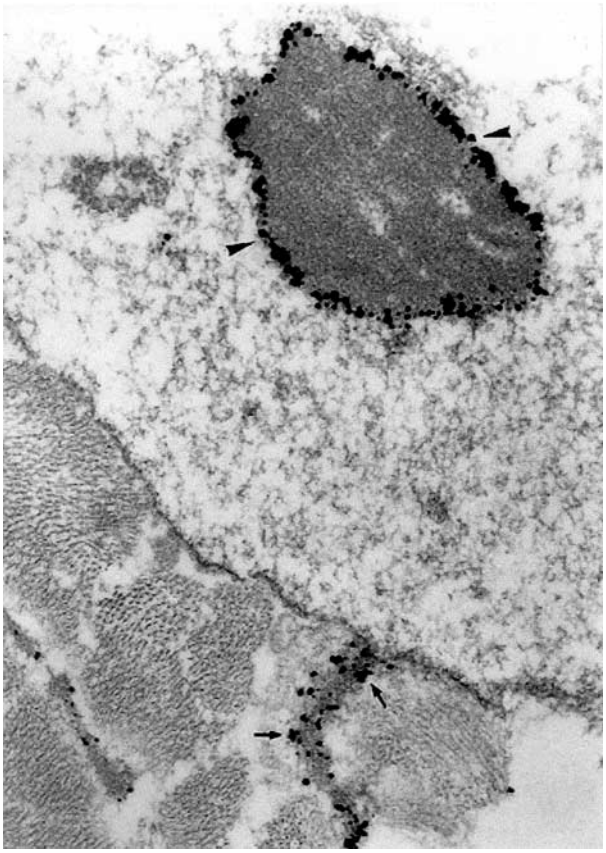

Figure 4. Intranuclear rod myopathy. α-actinin is labelled in sarcoplasmic Z-disks (arrows) and at the margin of an intranuclear rod (arrowheads), immunogold technique with silver enhancement. Magnification: ×31,500.

myopathy (19).

While mutations in patients with nemaline myopathy of the *TPM3* and *TPM2* genes appear to be rare — for instance, 76 families with nemaline myopathy have been found negative for TPM3 mutations (80) — mutational search for another actin-related protein gene, the nebulin gene, has identified a rather high frequency of mutations in families with nemaline myopathy, *i.e.* 41 out of 45 from several countries (91). By linkage analysis, the chromosome locus on 2q21.2-22 with the marker D2S2236 was identified for the classical autosomal-recessively inherited type of nemaline myopathy, *NEM2* (64, 68, 91). Point mutations have been discovered in the nebulin gene, both in homozygosity as well as in compound heterozygosity patterns (68). In a homozygous nonsense mutation in exon 185, by application of the SH3 antibody at the C terminal domain of the giant nebulin protein in a respective muscle biopsy specimen, one out of five *NEM2* families suggested a truncation of the nebulin protein (68), whereas an antibody against nebulin simple repeats M176-M181 showed immunohistochemical labelling identical to that in normal controls in all nebulin mutant families (68).

Having discovered mutations in genes of the actin-related protein, *TPM3*, and nebulin, accumulation of large aggregates of filamentous actin in two unrelated children with nemaline myopathy, though largely with intranuclear rod formation (31, 36, 38), resulted in the discovery of numerous missense mutations in the *ACTA1* gene for sarcomeric actin (65). The number of such missense mutations has now been enlarged to 25 (44). When testing parents of affected children with this *ACTA1* gene mutation, it was found that none of the parents was affected identifying these mutations as *de novo* mutations. Furthermore, these mutations are all isolated ones. The same Val163Leu mutation may result in clinically different forms: a rather severe fatal form as well as a much more benign form in a now 8-year-old boy with minimal weakness only (31, 36, 65). The large number of these isolated missense mutations in the *ACTA1* gene suggests a high vulnerability to mutational events of this gene.

As regards the association of actin aggregrates in nemaline myopathy, three groups of patients have emerged: (1) those having, occasionally, largely intranuclear rods and aggregates of actin (31, 65); (2) those apparently lacking rods, but having aggregates of actin (31, 65); (c) those with rod formation only, apparently lacking aggreates of actin (65). Biopsied muscle tissues showing only one of the components, *i.e.* either rods or aggregates of actin, may reflect errors in sampling.

Other families with nemaline myopathy have apparently been excluded from having known mutations, attesting to further affected genes in nemaline myopathy.

Immunohistochemistry, combined with electron microscopy, has fruitfully been employed in morphological studies on nemaline myopathy, successfully verifying the nemaline nature of intranuclear inclusions (Figure 4) (36, 38) as well as identification of thin filament aggregates in actinopathy as actin (31). These studies have opened new avenues to research concerning other protein components and their possible pathology in these nosological lesions, i.e. rods and aggregates of actin.

Central Core disease

Cores are well-demarcated but not membrane-bound mitochondria-free foci occurring in type I fibers which appear as clear circular zones in cross-sectioned myofibers (Figure 5). The finding of cores in a number

of myofibers of a given biopsy does not necessarily mean that the patient is afflicted with central core disease: 5 in 13 patients with biopsies demonstrating central cores were asymptomatic (75). A distinction is made between *central cores* and *minicores*. Minicores are smaller specimens of core lesions. They often arise multiple ("Multicores"). Minicores may occur in every pathological condition and even in normal controls. A genetic defect has not been found. However, a recent multicenter approach including 38 patients identified phenotypically homogeneous subgroups that could serve as a basis for the genetic approach (25).

Molecular genetics. CCD is an autosomal dominant disorder with variable penetrance. The disease has been linked to RYR1, the ryanodine receptor predominant in skeletal muscle. The RYR1 protein forms an elaborate tetrameric structure that acts both as a "foot" structure bridging the gap between the SR and the t-tubule in skeletal muscle, and a calcium release channel. The channel releases calcium into the cytoplasm in response to depolarization of the sarcolemma, providing a mechanism for excitation-contraction coupling. The human RYR1 gene is located on chromosome 19q13.1. The RYR1 protein is one of the largest known proteins with 2200 kDa corresponding to 5000 amino acids encoded by 106 exons (69). To date, seven missense mutations leading to CCD have been described (reviewed in 56). Most mutations are located either in the cytoplasmic N terminus or in a central cytoplasmic region. However, a mutation in the C-terminal region of the RYR1 protein, which forms the calcium release channel, caused an unusually severe and highly penetrant form of the disorder (53). Most patients afflicted with central core disease are susceptible to malignant hyperthermia (MH), but not all of them (15). However, there is genetic heterogeneity of MH and CCD. Curran and co-workers (15) reported on a family with a clear lack of segregation of CCD with chromosome 19 markers. Furthermore, a missense mutation in the β-myosin heavy-chain gene has been reported to cause central core disease in hypertrophic cardiomyopathy (22). An Arg163His mutation conferred CCD to one individual but not to his daughter carrying the same mutation (54). In view of the genetic heterogeneity it is recommended at the time being to perform an in vitro contracture test for all putative CCD cases (15). A hypothesis for the possible relationship between disturbed Ca^{2+} release and the building of central cores has been offered (52). This hypothesis is based upon the assumption that plasma membrane Ca++ pumps and Na+/Ca++ exchangers efficiently protect the periphery of a myofiber from elevated Ca2++ concentrations, whereas in the core of myofibers, mitochondria participate in removal of excess Ca++ from central areas of the fibers, thereby destroying themselves, resulting in mitochondria-free cores (52). Interestingly, the number of central cores in a given biopsy is independent of the severity of the disease (15).

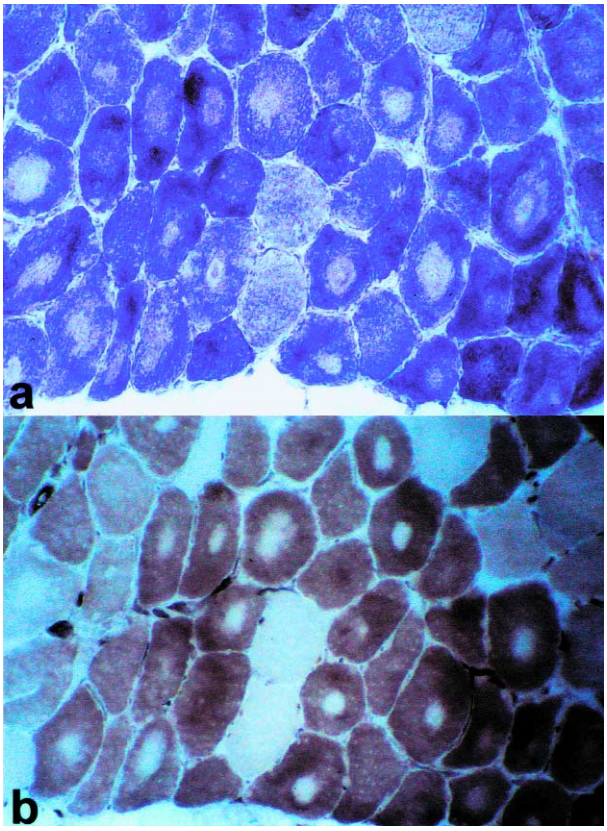

Figure 5. Central core disease. (**a**) Central cores in type I fibers. NADH tetrazolium reductase. (**b**) Cores are sometimes visible in myosin ATPase preparations (acid preincubation, pH 4.2). Magnification ×100.

Congenital myopathies with central nuclei: Centronuclear myopathy and X-linked myotubular myopathy

General comments on central nuclei. The muscular structures which contain myonuclei in a central position are usually called myotubes. They occur transiently in normal myogenesis during fetal development and also in adult muscle after necrosis of a myofiber has occurred with ensuing regeneration. Central nuclei are not found in normal postnatal muscle except in the vicinity of myotendinous junctions. This might be a reaction to

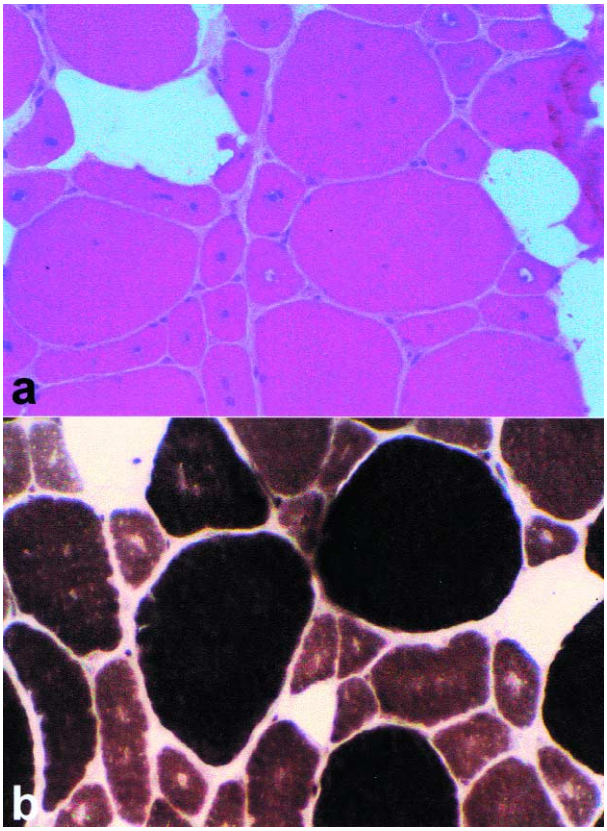

Figure 6. Centronuclear myopathy. (**a**) Internal nuclei both in small and large fibers. (**b**) Type I fiber smallness. Myosin ATPase, pH 4.2. Magnification ×200.

mechanical stress to which myofibers are subjected at the myotendinous junctions. Centronuclear myopathy (CNM) and myotubular myopathy (MTM) are characterized by central nuclei although necrosis and regeneration is not a typical feature. The two diseases are two separate clinico-pathological and genetic entities.

Centronuclear myopathy

CNM usually runs a mild course, but the clinical course is not benign for some patients: Respiratory or cardiac failures by 20 years of age occurred in a series of 55 patients (1). Inheritance is considered to be autosomal recessive, but occasional autosomal dominant cases occur, and many cases are sporadic. Some patients present their first symptoms when they are adults. The gene defect has not been detected yet. The morphological picture is characterized by an abundance of central nuclei and type I fiber predominance or even type I fiber uniformity, sometimes in conjunction with type I fiber smallness (24) (Figure 6). Although a previous report favored an arrest in the normal migration of the nuclei from the center to the periphery of the muscle fibers during fetal myogenesis (79), several recent reports showed that the centralization of the nuclei had occurred postnatally (discussed in 93). The pathogenesis of the type I fiber predominance has not been elucidated.

X-linked congenital MTM

Most patients die from respiratory failure at a mean age of 4-5 months, but long-term survivors up to 54 years have been described (reviewed in 48). About one third of all MTM cases are sporadic (81). The myofibers in MTM resemble myotubes (Figure 7a). In effect, the notion that the morphological pattern is due to arrested maturation during fetal myogenesis (73) has not been challenged yet and is now thought to be due to failed secondary myogenesis (47), perhaps due to incorrect dephosphorylation of phosphatidylinositol 3-phosphate (83). However, formation of myotubes occurs normally in cultured satellite cells from patients afflicted with X-linked MTM (85). The disease is always present at birth.

Molecular genetics. X-linked MTM is caused by a mutation of a gene which is localized to the long arm of the X chromosome (Xq28) (47). The protein encoded by the MTM1 gene was designated myotubularin (47) (Figure 7b). Moreover, the protein shares a high amount of amino acid identity with related (MTMR) proteins (47). None of the MTMR proteins has so far been shown to be mutated in MTM, but MTMR2 has recently been shown to cause the hereditary neuropathy Charcot-Marie-Tooth type 4B (6). The myotubularin gene is 100 kb in length and consists of 15 exons, and encodes for a 603 amino acid protein (82) (Figure 7b). It contains the consensus sequence for the active site of protein tyrosine phosphatases (81). The protein has been found to act on the phosphatidyl-inositol-3-phosphate pathway (5). Myotubularin is localized to the nucleus of transfected cells (14) but wildtype myotubularin was found to be localised in the cytoplasm in transfection experiments performed on a large variety of cell lines (5). Furthermore, there is evidence that myotubularin is localized to the cytoplasm of C2C12 mouse myoblasts (83). The MTM1 gene is ubiquitously expressed in all human tissues, however, a smaller transcript, the expression of which is restricted to skeletal muscle and testis, has also been identified (47). 133 different mutations have been detected so far (48). The mutations are distributed along the entire length of the MTM1 gene, with exons 4, 5, 8, 9, 11, and 12 containing more than two-thirds of reported mutations (82). Among the manifold gene mutations found in MTM are large deletions, non-

sense, frameshift, missense, and splice-site mutations and intronic variants causing partial exon skipping (82). Some genotype-phenotype correlations are worth mentioning. All missense mutations affecting the phosphatase active site or another domain, the SET interacting domain (SID) domain, appear to be associated with a severe phenotype, whereas a patient with a deletion in exon 15 had a mild clinical course and was still alive at 42 years, suggesting that this exon represents less vital regions of the gene (reviewed in 48). However, there is a phenotypic variability within some families in spite of affected members all sharing the same genotype (9). Carrier detection and prenatal diagnosis is possible. An affected fetus has been identified as early as the 13th gestational week by linkage analysis (50), which was later confirmed by direct mutational screening (81). The clinical and histological diagnosis of X-linked MTM has to be confirmed by molecular analysis of the MTM1 gene. This is important because phenocopies exist, the most obvious one being the severe neonatal form of myotonic dystrophy (discussed by Brown *et al*., this volume).

Vacuolar myopathy

The term vacuolar myopathy is a descriptive one and denotes vacuoles within muscle fibres as a disease-related and even disease-specific feature. Vacuolation of the sarcotubular system is typical of hypokalemic periodic paralysis, now called sodium channellopathy. Lysosomal vacuoles are a hallmark of lysosomal diseases, of which type-II glycogenosis is a major neuromuscular disorder.

Recently, vacuolar myopathies have also been identified among congenital myopathies. Currently, there are two types genetically defined, both being X-linked inherited (2). One is marked by autophagy and redundant basal lamina of muscle fibres. It is a pure muscle disorder marked by weakness of proximal limbs (43, 87). Numerous autophagic or rimmed vacuoles are encountered within muscle fibres, often close to the sarcolemma. Débris from such autophagic vacuoles may be encountered outside of the sarcolemma in the extracellular space. The gene for this X-linked myopathy (XMEA) seems to be located at Xq28, telomeric to the marker DXS1193 (88). No gene product has yet been identified.

The other X-linked vacuolar myopathy has been described in conjunction with cardiomyopathy and mental retardation (61). These vacuoles are often lined by plasmalemma and basal lamina suggesting invaginations deep into the muscle fibres where even extracellular components such as collagen may be identified. The gene of this X-linked myopathy has been mapped to Xq24, encoding a lysosome-associated membrane protein B or LAMP-2 (63).

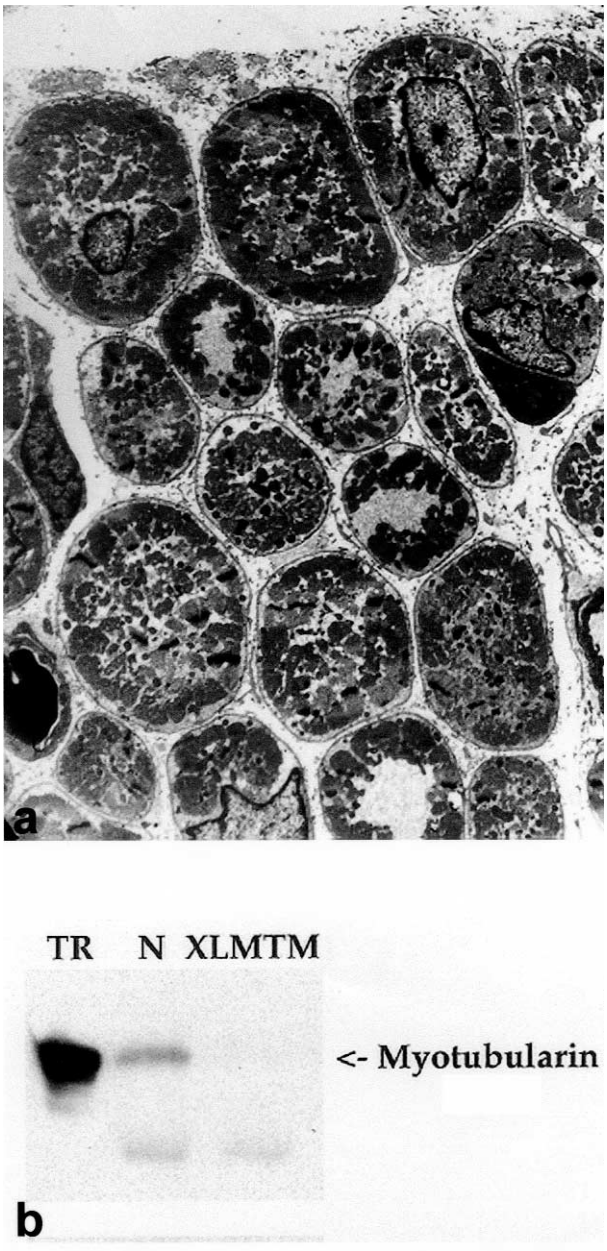

Figure 7. Myotubular myopathy. (**a**) Electron micrograph. A few myotubes have central myofibril-free zones. Magnification ×1000. (**b**) Western blot of myotubularin. TR: Transformed cells. N: Normal control. The lower band is a myotubularin degradation product. Gift from Dr. Jocelyn Laporte, Strasbourg, France.

Whether either one of these two X-linked vacuolar myopathies is identical or similar to Danon's myopathy, *i.e.* lysosomal glycogen storage disease with normal

acid maltase (17, 41, 71, 84) remains to be seen. Clinically, Danon's disease resembles X-linked vacuolar myopathy on Xq24.

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