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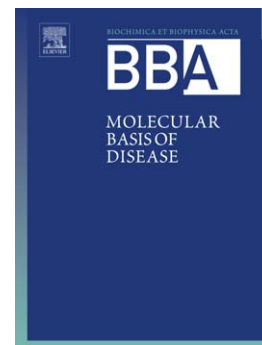
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Molecular biology of distal muscular dystrophies - sarcomeric proteins on top

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Abstract

During the last 10 years several muscular dystrophies within the group of distal myopathies have been clarified as to the molecular genetic cause of the disease. Currently, the next steps are carried out to identify the molecular pathogenesis downstream of the gene defects. Some early ideas on what is going on in the muscle cells based on the defect proteins are emerging. However, in no single distal muscular dystrophy these efforts have yet reached the point where direct trials for therapy would have been launched, and in many distal dystrophies the causative gene is still lacking. When comparing the gene defects in the distal dystrophies with the more common proximal muscular dystrophies such as dystrophinopathies or limb-girdle muscular dystrophies, there is a striking difference: the genes for distal dystrophies encode sarcomere proteins whereas the genes for proximal dystrophies more often encode sarcolemmal proteins.

Introduction

Distal dystrophies – distal myopathies are defined as inherited primary muscle disorders characterized by progressive muscular weakness and atrophy beginning in the hands, forearm, lower legs or feet [1]. Distal myopathy has prevailed as the main term for historical reasons even if the disorders in a clinical sense are muscular dystrophies.

The different entities of this category have all first been determined as new disorders based on their clinical findings (Table 1). During the last 10 years, the molecular genetic background of the different entities has been resolved in most of the major forms. This significant progress has shown that many gene defects underlying distal dystrophies may cause not only a distal presentation but a variety of clinical phenotypes [1]. Eventually, when most myopathies/dystrophies have been defined by their gene defect, distal myopathy as a category of classification may become less important. In the future, the currently emerging molecular genetic definition may in turn be replaced by categories defining distinct molecular biological functions and pathways disrupted by the gene defects.

The first well documented large family published as a distal myopathy was reported in 1943 [2]. Six patients in a dominant family with 12 affected males had distal leg weakness with onset in early adulthood. Later studies in this family showed desmin accumulations in muscle biopsy and a mutation in the desmin gene [3]. The change of classification in this family, from distal myopathy to desminopathy, illustrates current developments following progress in molecular genetics.

In this review the traditional grouping of the different entities based on clinico-genetical presentation has given way to a new molecular biology type of basic approach in order to facilitate the association of the basic clinical problem with the biological and functional units of the muscle cell. However, our current understanding of the molecular

pathophysiology of these disorders is still highly incomplete and only emerging. Many defects involve large sarcomere proteins and hypotheses on how these defects could be overcome by therapeutic interventions are, at present, very vague. In disorders with dominant gain of function and abnormal accumulations of protein, silencing the mutant gene with small interfering RNA would theoretically look like an option.

Distal muscle weakness and atrophy is sometimes the presenting symptom and sign in myopathies that are mainly characterized by other major findings. These conditions are noted only briefly. The basic molecular biology of dysferlinopathy is covered in the LGMD-review and GNE-associated hereditary inclusion body myopathy (hIBM) is presented in the corresponding review.

Distal dystrophies caused by defects in structural components of the sarcomere

Defects in the excitation-contraction coupling machinery of the A-band part of the sarcomere generally cause cardiomyopathy (personal comment). Genes and proteins involved include myosin heavy and light chains (MHC and MLC proteins), myosin binding protein C (MYBP-C) and titin. However, if the defects lie outside the A-band region of these proteins, other phenotypes will develop (Fig 1A).. Also mutations in proteins or particular parts of proteins in the Z-disk and I-band of the sarcomere may cause cardiomyopathy.

Some distal dystrophies are very early onset, such as myosinopathy, whereas others are late onset or even very late onset, such as titinopathy or myotilinopathy. In the early onset forms the defect in target muscles is possibly congenital although clinical signs, such as drop foot, are usually observed when patients start to walk. This type of very early onset of muscle weakness is suggestive of developmental/constructional abnormalities in contrast to the late

onset disorders, which develop after long time usage as a deficiency of maintenance and an imbalance between damage and repair.

Thick filament disorders – myosinopathies

Mutations located in the tail region of the slow myosin heavy chain gene *MYH7* are the cause of early onset dominant distal myopathy (Laing myopathy) [4,5] (Fig 1A). A number of mutations have been reported in this region between residues 1500 to 1729, with preference for deletions or insertions of lysine residues or single residue changes introducing a proline in the alpha-helix. For example, the K1617del mutation has occurred independently in different populations and also ‘de novo’ in one of the studied families, forming a hot spot mutation [6]. Moreover, this location in the tail of slow MHC includes the domain defined to interact with myomesin and it is just N-terminal of the defined domain (between residues 1815-1831) for interaction with c-terminal titin [7]. The tails of individual MHC molecules are physically located in the M-line of the sarcomere (see below distal titinopathy), which is of interest regarding the phenotype, but the exact functional molecular consequences of the mutations in this part of the myosin filament are not known. Muscle fibers in affected muscles undergo atrophy more than fiber necrosis and type 1 fibers, besides atrophy, show reprogramming towards hybrid expression of different myosins [6]. Mutations in the ultimate c-terminus of slow MHC cause another phenotype: hyaline body myopathy, with variable clinical presentation and with unstructured myosin deposits in the hyaline bodies in the myofibers [8]. Mutations in the proximal neck - head part and different mutations in the rod domains of slow MHC may cause cardiomyopathy [9,10].

Clinical phenotype

Clinically distal myosinopathy is to some extent similar to the first ever reported distal myopathy; Gowers’ description of a patient in 1902 [11,12]. Weakness begins in the

lower leg anterior compartment between 1 and 25 years of age with a characteristic hanging big toe-sign and severe atrophy and weakness of neck flexors, the sternocleidomastoid muscles (Fig. 2A). The disease progression is slow, eventually resulting in weakness of finger flexors, shoulder, trunk, facial and tongue muscles. Scoliosis and tendon contractures, mainly in the ankles, are common. Cardiomyopathy has rarely been described [13], and severe respiratory problems have not been encountered.

Laboratory findings and muscle pathology

Serum CK is slightly elevated or normal, and EMG shows early-recruiting short duration, low amplitude and polyphasic motor unit action potentials. Spontaneous activity was found in the more severely affected muscles, without signs of neuropathy [12]. Muscle imaging is highly suggestive of the diagnosis showing, besides overall small muscle mass, severe changes of degeneration in the anterior tibial muscles (Fig 2B) and, at advanced age, also lesions in posterior calf and the proximal thigh muscles. The early atrophy of sternocleidomastoid muscles can also be captured [6].

On muscle biopsy rimmed or other vacuoles were found in a minority of the reported biopsies [6,14]. Findings in more affected anterior tibial (TA) muscle show very distinct pathology with bimodal fiber size distribution: large number of highly atrophic tiny fibers scattered and in groups in portions with end stage pathology, simulating large group atrophy. Practically all fibers express fast myosin, whereas normally only a small minority of fibers is type 2 fibers and 80-90% may be slow type 1 fibers in TA (Fig 2C-D). In distal myosinopathy almost all type1 fibers expressing slow myosin are highly atrophic group and they also express fast myosin, suggesting a complete reprogramming of fiber type specifications due to the slow myosin *MYH7*-gene defect. [6].

Thin filament disorders – nebulinopathies

The hallmark of defects in thin filament proteins actin, troponin, tropomyosins and nebulin is congenital myopathy, and even more specifically: nemaline myopathy [15]. Nemaline rods occurring in myofibers on light and electron microscopy is a diagnostic corner stone in the differential diagnostics of congenital myopathies, and if present, they direct molecular genetic search in the five genes encoding thin filament proteins known to cause nemaline myopathy. So far, there is no good pathophysiological explanation why thin filament proteins and not other sarcomere proteins cause this specific type of protein aggregates. Possibly some intrinsic property of the thin filament structure, lacking in the other filament systems, may account for nemaline type of aggregation if one of the basic components is defect and harms the correct turnover.

However, not all genetic defects in the thin filament proteins cause nemaline myopathy. We have recently identified recessive missense mutations in nebulin (*NEB*) gene which do not cause nemaline rods to occur in affected muscle cells, but they cause a recessive distal myopathy [manuscript in preparation]. Interestingly, exactly the same missense mutations induce nemaline rod pathology when they combine with truncating or splicing mutations in compound heterozygous patients, in the same way as with other than missense mutations on both chromosomes.

Clinical phenotype and laboratory findings

Mutations in nebulin are a well known cause of autosomal recessive congenital nemaline myopathy [16]. Besides generalized weakness and muscle atrophy of variable severity, the distal muscles are usually more involved than the proximal ones. In our distal nebulin myopathy patients with missense mutations on both chromosomes, the clinical and the myopathology phenotype is clearly different. Extensor muscles of hands and feet are severely affected, but the progression is very mild, walking is preserved and patients do not have

major disability in adulthood. Muscle imaging reveals selective degeneration in the anterior tibial muscles, EMG is myopathic and CK is normal or mildly elevated. Muscle biopsy shows scattered and grouped atrophic fibers. The reason for the disorder not to have been identified earlier as nebulinopathy is that the homozygous missense mutations do not produce nemaline rods on light microscopy, despite multiple biopsies in some cases (Udd 2006, personal communication). Careful re-analyses of semi-thin sections and electron microscopy in our patients with homozygous mutation in exon 155 revealed rare small rod type changes, but on routine light microscopy no rods could be identified. Homozygous missense mutation in exon 121 caused disintegration of sarcomere structure but absolutely no rods even on electron microscopy in multiple biopsies from the same individual.

Molecular diagnosis of this group of patients is currently laborious considering the large size of the gene.

Third filament system – titinopathies

The first identified genetic defect underlying autosomal dominant distal dystrophy was mutations in c-terminal titin that cause tibial muscular dystrophy (TMD, OMIM #600334, Udd myopathy) [17,18]. Titin is the largest single polypeptide protein in nature and each molecule spans over one half of the sarcomere from Z-disk to M-line, interacting both with thin filaments and thick filaments (Fig 1A-1B). During myofibrillogenesis titin is expressed before the other filament systems of the sarcomere and is thought to form a scaffolding ruler for the highly organized structure of the sarcomere [19, 20]. Titin interacts repeatedly with myosin filaments in the A-band region and through the interface of MYBP-C, causing strong positional fixation of the contractile system and keeping the myosin thick filaments always centered in the sarcomere [21]. In the middle of the sarcomere, in the M-line portion, titin has less modular and more complex sequence properties, including a kinase domain, interaction domains for myomesin and unique sequences with unknown functions [22]. In the M-line, c-

termini of titin molecules from both flanking Z-disks meet and probably overlap. Since n-terminal titins also overlap each other from neighboring sarcomeres in the Z-disk, titins in consecutive sarcomeres may form a continuous filament structure extending from one sarcomere to the other (Fig 1A-1B). Recently, we have identified M-line titin, i.e. the kinase domain (Fig 1B), as a sensor of mechanical contraction activity in the myofiber with a physically interacting signaling complex of proteins [23]. This signaling complex includes MURF2, which in the normal state of muscle activity stays in the complex in the M-line, but dissociates and shuttles to the nucleus where it inactivates the activity of the transcription factor SRF, if muscle activity is blocked chemically or by denervation[23]. M-line titin thus seems to have important signaling functions for regulation of transcription and turnover in relation to variable contractional demands. One point mutation in the regulatory tail of the kinase domain is able to totally disrupt the function of the signaling complex and causes a severe dominant muscle disease HMERF with respiratory failure [23].

Molecular genetics

In close proximity to the kinase domain in the periphery of the M-line is the ultimate c-terminus of the titin molecule. In its last domain, the Ig-like M10, encoded by the last exon of the gene, *Mex6*, we found the first mutations in TMD distal dystrophy (Fig 1B) [17]. The mutation in Finnish patients, *FINmaj*, is a complex in frame ins/del of 11 consecutive base pairs exchanging four amino acids in M10 [17]. The protein, however, is damaged beyond M10 since epitopes in the third last domain M9 are not recognized by specific antibodies [17]. The mutant titin is incorporated in the sarcomeres shown by immunohistochemistry with antibodies to different other parts of the protein in homozygous mutant muscle, and mutant titin seems to be intact up to the fourth last domain M8 (Fig 1B).

In two unrelated French families a point mutation changing a lysine to proline was found in the same last *Mex6* exon [19]. Later a third mutation in the same last exon was found in a

Belgian TMD family [24], and more unpublished mutations, also in the last Mex6 exon, have been identified in Spanish and other French families (Udd, personal communication). These mutations are available for diagnostic testing. In new unrelated TMD patients searching for mutations by sequencing the last Mex 6 exon may be productive.

Molecular pathomechanism of c-terminal titinopathy - TMD

Even in homozygous mutant muscle the overall sarcomere structure is intact on electron microscopy, suggesting no major mechanical disruption caused by the mutations [25]. The downstream chain of molecular events is, however, not yet defined. It will be complex, because in the few rare patients with homozygous FINmaj mutation the phenotype is completely different: early childhood onset proximal limb-girdle muscular dystrophy with loss of ambulation around age 25 (LGMD2J) [26,27]. Whereas in TMD, the muscle involvement is focal and selective targeting first the tibial anterior muscles, in LGMD2J the dystrophy is generalized with replacement in all large muscles at the age when clinical symptoms from weakness of ankle dorsiflexion start to emerge in the heterozygous TMD [27]. This complete difference may suggest that downstream molecular pathways are partly different in TMD vs. LGMD2J.

The only established ligand of c-terminal titin is muscle specific calpain-3, that is responsible for LGMD2A when mutated [28, 29]. In homozygous LGMD2J calpain-3 is severely reduced [30], but not in heterozygote TMD muscle, indicating that some other disrupted protein interaction may mediate pathology later in life in the heterozygotes. Myosin heavy chain (MYH7) tail region has been shown to interact with titin (see above), but the interacting region on titin is not defined yet.

Clinical phenotype

The clinical findings present after age 35, selectively involving the tibialis anterior

and, in advanced stages, the long toe extensor muscles [25] (Fig. 3 A). Weakness at onset may be asymmetric and progression is slow. Mild to moderate proximal leg muscle weakness occurs after age 70 in a minority of the patients, but patients rarely become wheel-chair bound even at advanced age. Clinically, the sparing of short toe extensors (extensor digitorum brevis) is an important finding to make distinction from neurogenic foot drop. Unlike in Welander distal myopathy (WDM), hand muscles are rarely affected in TMD [25]. Despite this characteristic presentation, a recent study of 207 mutation confirmed patients showed unexpected variants of the phenotype in 9% of the patients [25].

Epidemiology

Recent studies have shown a high TMD prevalence in Finland of more than 8/100,000 [27,31]. Patients are found all over the country, although the origins of their families can be traced back to the west coast region of middle Finland and to the Savo-Karelia area of eastern Finland [25]. TMD has also been identified in Sweden, Norway, Germany and Canada in descendants of Finnish immigrants. Moreover, TMD families have recently been identified in other populations without connections to the Finnish background and with other mutations in c-terminal titin in France, Belgium and Spain [24,32] (Udd personal communication 2006), and there is no reason to believe that titin mutations should be restricted to certain populations.

Laboratory findings and muscle pathology

Patients with TMD have normal or mildly elevated CK [25]. In affected muscles EMG studies showed low-amplitude, short-duration motor unit potentials on moderate activity [26]. Increased insertional activity, frequent fibrillation potentials, and occasional high-frequency and complex repetitive discharges at rest may be obtained in clinically affected muscles. In clinically unaffected muscles of the upper limbs polyphasic potentials

may be recorded [25].

CT and MRI provide accurate data on the selective involvement of individual muscles in TMD. Changes of fatty degeneration appear at the time of clinical weakness [33]. The evolution of selective involvement over time is very distinct in TMD. Together with the presenting weakness of ankle dorsiflexion, fatty degenerative changes appear in the anterior tibial muscle. After 10-15 years of symptoms, lesions usually appear in the long toe extensor muscles, and in hamstring, gluteus medius, and tensor fasciae latae muscles [33] (Fig. 3B). Initially, the involvement may be asymmetric. Later, focal lesions may appear in apparently asymptomatic muscles, such as the soleus, when other unaffected muscles mask the loss of power and prevent the lesion from being observed clinically [33].

Muscle specimens in TMD reveal myopathic alterations, including variation of fiber size, thin atrophic fibers, central nuclei, structural changes within the fibers, endomysial fibrosis, and fatty replacement in the end stage muscle [34]. Necrotic fibers, some showing phagocytosis, are rare in TMD. Fiber type differentiation was normal, both major fiber types being equally involved in the pathological process (Fig 3C). Many rimmed vacuoles were acid phosphatase positive, while others were ubiquitin positive and, with rare exceptions, they were not lined by sarcolemmal membrane proteins. Congo red stains and immunohistochemistry for beta-amyloid and amyloid precursor protein were negative in TMD specimens containing fibers with rimmed vacuoles, in contrast to sporadic inclusion body myositis. Ultrastructural studies in TMD revealed overall well preserved sarcomere structure, even in the homozygote LGMD2J mutants. Focal cytoplasmic and sarcomeric degradation products and occasional tubulofilamentous inclusions were encountered in the vacuolated fibers [25]. Rimmed vacuoles in TMD are usually not membrane bound and thus do not fulfill the morphological criteria of autophagic vacuoles, even though the vacuolar space contains numerous small vesicles compatible with lysosomal autophagic components.

Telethoninopathy

The other name of this protein, T-cap, indicates its function as capping titin molecules in the periphery of Z-disks, and telethonin may thus be connected to the third filament functions rather than characterized by its location to the z-disk region (Fig 1).. Telethonin is shown to be a substrate of the serine-threonine kinase in c-terminal titin. In mature muscle these locations are physically distant, suggesting either shuttling of telethonin or phosphorylation regulation only temporarily during myofibrillogenesis when c-terminal titin and the kinase is located also in emerging Z-disk regions. No further molecular pathomechanisms are detailed on the effects of mutations in telethonin, which usually are reported to cause a proximal LGMD2G phenotype, but, in fact, many of the patients have more distal atrophies and weakness at onset [1]. The myopathology is myofibrillar rimmed vacuolar.

Defects in Z-disk proteins and distal muscular dystrophies

Recent progress has delineated several genes that underlie the muscle pathology characteristics of myofibrillar myopathy (MFM): desmin, α B-crystallin, myotilin, ZASP and filaminC (Fig 1A) [35-37]. Patients with dominant myofibrillar myopathy frequently present as distal myopathies [38-41]. There is a wide range of onset, patterns of muscle involvement, and progression rates. Patients may have additional features cardiomyopathy, respiratory muscle weakness, neurogenic findings and even EMG myotonia. The pathology includes accumulations of all these proteins irrespective of primary gene defect, suggesting that these share molecular pathways involved in actin dynamics organized by the Z-disk. Besides accumulations of these proteins, congophilic amyloid products of myofibrillar degradation and ectopic aggregation of dystrophin and gelsolin appear in abnormal myofibers [35].

Desminopathy

Desmin is a muscle-specific cytoskeletal protein and belongs to the type III family of intermediate filaments. Desmin mediates attachments between the Z-disks of parallel myofilaments and provides attachments between Z-disk and membrane-associated proteins to form a force-transmitting system (Fig 1A).. One of the earliest reports on distal myopathy described a very large autosomal dominant family with branches both in Europe and in North America [2]. This family later proved to have a mutation in the desmin gene [3]. What properties exactly are lost or gained by the mutations that cause desmin together with other Z-disk proteins to agglomerate in desminopathy is not known. Transfection studies of some mutations caused incorrect forming of desmin intermediate filament network, induced the collapse of a pre-existing desmin cytoskeleton, altered the subcellular distribution of mitochondria, and led to abnormal cytoplasmic protein aggregates [43].

Myotilinopathy

Myotilin (myofibrillar protein with titin-like Ig domains) is a 57 kDa Z-disk component that interacts with alpha-actinin [44], filamin-C, FATZ (calsarcin) [45], and actin [46] (Fig 1A).. It also controls sarcomere assembly [47]. Myotilin contains a serine-rich amino-terminal region (residues 28 to 124) with a hydrophobic stretch (residues 57 to 79), two Ig-like domains with considerable homology to certain Ig domains of titin (residues 252 to 341 and 351 to 441), and a carboxy terminal tail. The alpha-actinin binding site resides between myotilin residues 79 to 150, and the filamin-C binding site is located in the second Ig-like domain. Myotilin dimerizes via its C-terminal half, which may be necessary for the actin-bundling activity. Myotilin is strongly expressed in skeletal muscle and weakly in cardiac muscle [48]. Intramuscular nerve fibers are stained with myotilin antibody [49]. Myotilin is important for actin dynamics [47].

Molecular genetic findings

Several *TTID* missense mutations have been reported. In 2004, three novel mutations (S60C, S60F, and S95I) and the previously identified LGMD1A S55F mutation were reported in 6 unrelated patients with myofibrillar myopathy [35]. In a Spanish cohort of 13 patients, three of the known and two new missense mutations were identified [50]. All these 6 mutations are located in same region of the gene, the serine-rich exon 2 encoded domain, and many caused predominantly late onset distal dystrophy.

Considering that the pathology in the family reported by Penisson-Besnier et al. [51] resembled more that of myofibrillar myopathies, the myotilin gene was a good candidate in this family. Sequencing showed that a previously reported mutation S60F [35], segregated completely with the affected patients in the family and was not present in healthy individuals [52]. Interestingly, this is another muscle specific protein that in some instances may cause a dystrophy with a predominant proximal LGMD-phenotype (LGMD 1A) [53] and in others a very pronounced distal affection [51].

Clinical example

Penisson-Besnier et al. described an autosomal dominant French family with a very late onset of distal myopathy around the age of 60 years [51]. Weakness and atrophy was more severe in the posterior calf muscles than in the anterior lower legs muscles at onset. Later the patients experienced progression to proximal and upper limb muscles, although with retained walking ability even into later age. Cardiomyopathy was not observed in the patients.

Laboratory findings and muscle pathology

Muscle enzyme CK was either normal or mildly elevated. EMG showed myopathic changes with fibrillations and complex repetitive discharges. Muscle imaging confirmed the

clinical findings of extensive involvement of calf muscles showing dystrophic fatty replacement and very mild proximal leg muscle involvement. Histopathology of proximal upper limb muscles showed a variety of pathological findings including multiple large non-rimmed vacuoles, focal sarcoplasmic desmin reactive masses that stained darker on Gomori trichrome, rimmed vacuoles, and IBM-like cytoplasmic and nuclear filaments on electron microscopy [51]. Immunohistochemistry revealed abnormal myotilin aggregations and less pronounced ectopic expression of desmin, ZASP and aB-crystallin in the same aggregations. [52]

Zaspopathy

ZASP (Z-disk alternatively spliced PDZ-domain containing protein) is a component of the Z-disc and has known interactions with alpha-actinin (Fig 1A) [36,54]. Further mechanisms by which mutated ZASP causes such widespread disintegration of the sarcomere structure are so far elusive, but most mutations identified to cause MFM pathology are located in the Zasp-like motif encoded by exon 6, which is expressed in skeletal muscle and primarily responsible for the alpha-actinin binding [54,55]. Mutations in the alternatively expressed cardiac exon 4 are shown to cause cardiomyopathy [56]. Studies on mouse models including knock-outs suggest that ZASP is required for striated muscle function and maintenance during the stress of contraction, but not for initial formation of the Z-disk [57].

Clinical example

In 1974, an autosomal dominant family (Markesbery-Griggs) with six patients affected by distal myopathy was reported [58]. Weakness started in the anterior distal leg muscles between 43 and 51 years of age, then spread to the intrinsic hand and wrist extensor muscles, and eventually to the proximal limb and trunk muscles. Facial, bulbar or respiratory muscles were not involved. The disease progressed slowly, did not alter the length of life, but caused

loss of ambulation in senescence. Cardiomyopathy was present in one of the patients.

Molecular genetics

Early linkage studies in this classical Markesbery-Griggs family indicated the disorder could be linked to the titin locus 2q31 [59]. Extensive mutation search in the huge titin gene was not productive and no mutations were detected. With the reports of myotilin and ZASP mutations in subsets of myofibrillar myopathy patients [35,36], these genes became candidates, supported by the fact that muscle pathology had similarities to myofibrillar myopathy. Sequencing of myotilin gave normal results, whereas the previously reported C523G mutation A165V in ZASP was identified as the cause of disease in the family. (Griggs et al paper in progress 2006)

Laboratory findings and muscle pathology

Patients have normal or only slightly elevated CK [58]. EMG studies showed low-amplitude, short-duration motor unit potentials on moderate activity. Most patients have increased insertional activity, frequent fibrillation potentials, and occasional high-frequency and complex repetitive discharges at rest [58].

Muscle biopsies reveal myopathic alterations, including variation of fiber size, central nuclei, structural changes within the fibers, endomysial fibrosis, and fatty replacement of muscle [58]. Necrotic fibers, some showing phagocytosis, and fibers with rimmed vacuoles are abundant. In addition, some fibers contain single or multiple non-rimmed vacuoles whose contents fail to stain for lipids or polysaccharides. Focal accumulation of homogeneous, granular material blue-red with Gomori trichrome was prominent in some fibers.

Immunohistochemistry showed excessive ectopic expression of myotilin with less similar changes in desmin, α B-crystallin and ZASP in the abnormal fibers. Early electron microscopy changes included dilation and vacuolization of the sarcoplasmic reticulum and streaming and

disruption of the Z-disk. Clumps of Z-disk material were found without the periodicity of nemaline rods [60]. Sarcoplasmic masses composed of glycogen granules, lipofuscin bodies, tiny vacuoles, degenerated myofilaments, Z-disk fragments, and myeloid figures were common. In retrospect these alterations are well compatible with reported changes in myofibrillar myopathy. The vacuoles are membrane-bound and contain osmiophilic vesicles, granular membrane structures, myeloid figures, and other products of cytoplasmic degeneration and fit the morphological criteria of autophagic vacuoles. They are frequently at the fiber periphery and sometimes covered only by the basement membrane indicating that they may undergo exocytosis.

Autopsy studies were conducted in two patients [58]. One patient had clinical evidence of a cardiomyopathy with cardiomegaly, congestive heart failure, and intractable tachyarrhythmias requiring pacemaker implantation. At autopsy there was diffuse interstitial fibrosis and diffuse degenerative change in cardiac muscle fibers consistent with a cardiomyopathy.

Mutation defects in membrane signal transduction and repair proteins

Caveolinopathy

Defect caveolin-3 is the molecular cause of LGMD1C. However, mutated caveolin-3 may also cause 'rippling muscle disease', and other phenotypes associated with caveolin-3 mutations include distal myopathy with pronounced atrophy of intrinsic muscles in hands and feet [61,62]. The molecular pathomechanism of caveolinopathy is not clarified. Based on the function of the caveolae on the sarcolemma, disruption of signal transduction through caveolae is thought to be the consequence of mutant caveolin-3 (Fig 1A).. *CAV3* mutations are dominant, implicating a negative effect of the mutant on the wild type copies of the

protein. Dysferlin seems to interact with caveolin-3, and dysferlin immunostaining is markedly attenuated in patients who harbor mutations in caveolin-3 [61,63].

Distal dysferlinopathy

Dysferlin is a sarcolemmal protein with expression also in the cytoplasm (Fig 1A). Dysferlin has not been found to have a role in the dystrophin associated protein complex, although all its functions are not yet known [64]. Dysferlin, by analogy to FER-1 gene in *C. elegans* and by having calcium-binding C2 domains, may have a role in membrane fusion and maintaining the structural integrity of the sarcolemma [64]. Dysferlin interaction with caveolin-3 suggests, that one function of dysferlin may be to subserve signaling functions of caveolae [63]. Secondary calpain3 defects have also been shown in patients with dysferlinopathies [65], but the interaction of calpain-3 with dysferlin remains to be clarified. New reports demonstrate a cytoplasmic localization of dysferlin and association with T-tubule system, resulting in co-precipitating of dysferlin with dihydropyridine receptor together with caveolin-3 [66].

Molecular genetic findings

The two phenotypes of dysferlinopathy: distal Miyoshi Myopathy (MM) and LGMD2B occurred with the same mutation in different individuals even within the same family [67-71]. Why the identical homozygous mutation may cause different phenotypes within the same family is not clarified. Numerous different mutations have been reported and they are widely distributed along the large gene. Newer methodology for mutation screening using cDNA from muscle has been described [72].

Not all patients with MM-like phenotype have dysferlinopathy. In four families with adult and later onset of symptoms, linkage to the MM locus 2p was excluded [73]. In two of these families a genome wide screen suggested linkage to a locus on chromosome 10 (LOD

2.57). One family was unlinked to both loci. Thus, the phenotype is apparently genetically heterogeneous, and MM might be reserved for the dysferlin mutated form, whereas others may be termed MM-like phenotypes until their molecular etiology has been clarified.

Molecular pathogenesis

Recent ultrastructural studies indicate numerous submicron sized defects in the plasma membrane, replacement of the plasma membrane by small vesicles, frequently disintegrating small papillary projections, as well as numerous small subsarcolemmal vacuoles as early events in the pathology [74]. These findings support concepts on the role of dysferlin in maintaining muscle fiber surface membrane integrity and resealing of continuous minor disruptions of the sarcolemma in muscle activity. Dysferlin apparently serves multiple functions and a tempting explanation for the variable clinical phenotype at onset of the disease would be that individual genetic backgrounds modify which pathway of dysferlin is more or less affected. Later in the disease course both the distal and the LGMD presentations merge and have similar outcomes.

Clinical findings

In 1977, two sporadic cases of distal myopathy with onset at the age of 19 and 20 years were reported from the USA [58], and Miyoshi et al. described a series of similar patients in Japan [75]. All patients had weakness and atrophy starting in the distal lower extremities, particularly in the calf muscles (Fig. 4A). First symptoms were difficulty in climbing stairs, walking briskly, or running, and inability of patients to hop on one leg [76, 77]. Proximal muscles were only minimally affected at the onset, as were hand muscles. Distal dysferlinopathy (MM) shows slow progression towards proximal muscle involvement and after 10 to 20 years of disease duration there are similarities with the evolution of the LGMD2B phenotype. Dysferlinopathy patients presenting with anterior tibial rather than with

calf muscle weakness have also been reported [78], but imaging studies of these patients revealed marked fatty degeneration also of the posterior gastrocnemius and soleus muscles [79].

Epidemiology

Since molecular diagnosis for dysferlinopathy became available by immunohistochemistry and immunoblotting using anti-dysferlin antibodies, and by direct DNA genetic analysis, patients with MM have been diagnosed in many different populations [73,80-83]. The disorder seems to occur worldwide with a frequency of about 1-2/10⁶.

Laboratory findings and muscle pathology

MM patients have very high serum CK values 10-100 fold the upper normal level [76,77,80], and this high leakage of CK is present even at preclinical stages [76]. EMG shows increased insertional activity, fibrillation potentials and abundant small motor unit potentials with early recruitment [75-77]. Muscle imaging with CT or MRI give clear insight in the involvement of different muscles during the disease process, and can also be used for assessment of individuals at risk (Fig. 4B).

Muscle biopsy shows nonspecific myopathic/dystrophic features with abundant necrotic fibers, regenerating fibers, abnormal variation in fiber size, and later connective tissue increase [76]. In many dysferlinopathy biopsies inflammatory infiltrates have been observed [84], and dysferlinopathy has been confused with polymyositis on many occasions. Immunohistochemistry and immunoblotting with anti-dysferlin antibodies showing absent dysferlin has become the gold standard for pathological assessment and diagnosis. [74]. Immunoblots of MM muscle extracts accurately reveal dysferlin deficiency, whereas immunostaining of cryostat sections of MM muscle is less reliable. Currently available anti-dysferlin antibodies may stain the normal muscle surface only weakly, even at high

concentration. On the other hand dysferlin may be non-specifically over-expressed in regenerative fibers in any myopathic disorder (personal comment).

Distal muscular dystrophy caused by sarcoplasmic enzyme defects

GNE-mutated distal myopathy with rimmed vacuoles (DMRV)

GNE is a dual epimerase/kinase enzyme and the rate-limiting enzyme in the sialic acid biosynthetic pathway [85]. (see the corresponding Chapter for GNE-details).

Molecular genetic findings

After the gene was identified in autosomal recessive quadriceps sparing myopathy/hereditary inclusion body myopathy AR-hIBM, DMRV in Japanese patients proved to be caused by mutations in the same *GNE*-gene, confirming these are allelic disorders [86]. Many different mutations in *GNE* have been reported in Japanese DMRV patient [87,88], and one of them, V572L, is more common and accounts for a founder effect [86].

Molecular pathogenesis

Mechanistic explanation of why the double enzyme defects cause rimmed vacuolar myofibrillar degeneration is still lacking. However, defect sialylation is one major hypothesis and experimental cell studies have shown encouraging results in reverting the phenotype by adding sialic acid (Nishino personal communication). Further experiments are ongoing including mouse models. For more details, see further the Chapter on hIBM.

Clinical findings

Distal myopathy patients with onset in early adulthood of weakness in the lower legs, typically in the anterior compartment, causing foot drop were reported by Nonaka et al. [89]. Progression to posterior compartment and proximal muscles was always present and major

disability usually was the outcome after 10-15 years of disease duration. Intrinsic muscles were also involved. DMRV followed an autosomal recessive inheritance pattern.

Laboratory findings and muscle pathology

CK values are normal or slightly elevated in DMRV patients and EMG shows abundant small motor units potentials and spontaneous discharges including positive sharp waves and fibrillations [89,90]. By definition DMRV is characterized by rimmed vacuoles in muscle fibres [90,91]. Necrotic fibers may or may not be present. Ubiquitin expression was increased suggestive of increased non-lysosomal degradative activity as another part of the degradative pathogenesis [92, 93]. Finally, tubulofilamentous inclusions like those occurring in sporadic IBM were also observed in DMRV [94]. These morphological studies were mostly done before the gene defect in DMRV was known and, thus, it is not known whether all studied patients were *GNE*-mutated cases.

Distal dystrophies with unknown molecular defects

Welander distal myopathy

In her thesis in 1951 Welander described 249 patients with this disease [95]. The initial symptoms were clumsiness in precise finger movements, beginning as extensor weakness in the thumb or index fingers and spreading to the other fingers. Distal leg involvement occurred later, with stumbling, difficulty in walking, and, eventually, inability to stand on the heels or development of a steppage gait. In a minority symptoms may start in the lower limbs. These early clinical observations have been documented 50 years later by muscle imaging [96]. Coldness of the hands and feet occurred in 90 percent of patients and the disease shows autosomal dominant inheritance.

The possibility of a neurogenic component in WDM was first considered by Welander

[95]. Mild neurogenic features were later detailed by Borg and coworkers who found abnormalities of sensory function and sural nerve histology [97], but no abnormalities of sensory nerve conduction velocities, nor any neurogenic findings in 8 anterior tibial muscle biopsies in young and middle-aged adults with early symptoms [98].

Epidemiology

There are no exact studies on epidemiology of WDM, but estimates based on patient numbers at different regional neuromuscular centers indicate a prevalence about $5/10^5$ in Sweden (Edström, personal communication). Recent data emerged from Finland where WDM has been identified in several families [99].

Molecular genetic findings

Genetic studies in well defined WDM families determined linkage of the disease to a locus on chromosome 2p13 close to, but outside the *dysferlin* locus [100,101]. All known genes in the linked locus have been sequenced without identification of the causative gene [102]. All WDM patients in Sweden and Finland carry the same haplotype at the locus indicating one single common founder mutation [99,100]. In one of the families included in the linkage studies, one patient was homozygous for the linked haplotype and he, indeed, displayed a much more severe myopathy compared to typical WDM [100].

Laboratory findings and muscle pathology

Serum CK may be normal or up to a threefold elevated in WDM patients [103]. EMG studies in WDM patients show brief, short and polyphasic motor unit potentials, and in severely affected muscles a decreased number of motor unit potentials. Spontaneous electrical activity at rest, fibrillation potentials, and discharges have been reported [103]. Computed tomography (CT) and magnetic resonance imaging (MRI) both show fatty

degeneration of affected muscles with replacement lesions in the anterior compartment and frequently in the soleus and gastrocnemius muscles in the leg [96,104].

In the affected muscles rimmed vacuoles were found in atrophic fibers of both fiber types. On electron microscopy tibialis anterior specimens revealed autophagic vacuoles harboring dense bodies, myelin figures, and tubulofilamentous inclusions [97,98].

Other distal myopathies - single families

Several single families have been reported with distal myopathy distinct to the known entities as shown by linkage exclusion to established loci or by linkage assignment to specific new loci. These are comprehensively reviewed elsewhere [1]

Distal phenotypes in other myopathies

The disorders described above were all reported as distal myopathies. However, there are many disorders which can present with a distal phenotype that were classified or reported under different headings. These are important when discussing phenotypes versus molecular pathogenesis, and they illustrate the further development of molecular genetics serving as a new basis for classification of myopathies. Moreover, molecular genetics has disclosed allelic disorders or even identical gene defects that may present with highly variable phenotypes [27]. Because of noteworthy general aspects on molecular pathomechanisms and selective muscle involvement, two entities are separately notified.

Myotonic dystrophy type 1

Myotonic dystrophy type 1 (Steinert's disease) is worldwide the most common cause of a distal muscular dystrophy phenotype, not classified so because of the overt multi-system character of the disease. The molecular biological reason of why distal muscles are preferentially involved is not known. The repeat expansion underlying the disease alters the

splicing machinery of gene transcription. At present a dozen different genes are shown to be affected and the list may grow to hundreds or more, but which of the abnormally spliced genes are responsible the distal muscle involvement is unknown [105].

Oculopharyngodistal myopathy

The extraocular muscles are rarely affected in muscular dystrophy. The exceptions are mitochondrial muscle disorders such as PEO, and oculopharyngeal muscular dystrophy (OPMD) which is caused by a short repeat expansion in the *PABP* gene. Japanese families with late onset dominant distal myopathy and prominent oculopharyngeal weakness, in one associated with cardiomyopathy, have been reported [106,107]. In other families the trait seemed to be autosomal recessive [108,109].

Other disorders with occasional distal presentation

Rare instances of distal weakness have been described in chronic polymyositis [110]. Inclusion body myositis (s-IBM) often presents with anterior compartment leg or forearm weakness [111].

Patients with either facioscapulohumeral dystrophy (FSHD) or scapulooperoneal syndromes, presenting with anterior tibial muscle weakness before facial or shoulder weakness is evident, are not very rare and may cause diagnostic confusion. In FSHD isolated calf muscle involvement has been reported rarely. Mutation analysis is mandatory to identify 'sporadic' cases of FSHD [112]. Most recently, the increased expression of *FGRI* gene that follows the FSHD mutation, was shown to also alter splicing machinery [113]. In other disorders rare cases may have a distal presentation such as: centronuclear myopathy [114], debrancher enzyme deficiency myopathy (glycogenosis type 3) [115], phosphorylase b kinase deficiency [116], and lipid storage myopathies [117]. A distal phenotype can also occur secondary to nephropathic cystinosis [118].

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Figure legends

1. Schematic drawing of the structural proteins of the sarcomere and proteins involved in distal myopathies indicated (1A). Below in Fig. 1B the same schematic sarcomere structure detailed for titin and known mutations in c-terminal titin causing TMD titinopathy (Udd myopathy) disease (1B).
2. Legs of a 42 year old male patient with mutation in slow myosin heavy chain *MYH7* gene causing MPD1 myosinopathy (Laing myopathy), showing the 'hanging big toe' sign but clinically not much visible tibial anterior atrophy despite total loss of function (2A). CT scan of lower leg muscles in a 12 year old male patient with *MYH7* mutation showing total replacement by fat and connective tissue in anterior compartment muscles and also severe changes in soleus muscles bilaterally (2B). Serial sections of end stage pathology muscle tissue from tibial anterior muscle (same patient as 2B) showing just one atrophic muscle fiber expressing slow myosin (2C), and all other highly atrophic fibers express fast myosin (2D), where the proportions normally are the opposite in this particular muscle.
3. Lower legs of a 48 years old male patient with FINmaj mutation in c-terminal titin causing TMD titinopathy (Udd myopathy) showing clear atrophies of the anterior compartment muscles with subsequent prominent ventral edge of the tibial bone (3A). CT scans of lower leg muscles in a 62 year old female patient with FINmaj mutation showing selective total replacement of all anterior compartment muscles: tibialis anterior, extensors hallucis longus and digitorum longus, with normal appearance of the other lower leg muscles (3B). Hematoxylin and eosin stained tibial anterior muscle biopsy from early stages of the disease in a 42 year old female patient with FINmaj mutation showing large variation of fiber size, increased connective tissue and one atrophic fiber with rimmed vacuolar change (3C).

4. Legs of a 24 year old male patient with dysferlin mutation showing clinically atrophic calf muscles on the left side (4A). Low field MRI muscle imaging in 32 year old male patient with dysferlin mutations causing total replacement of posterior calf muscle bilaterally (4B).

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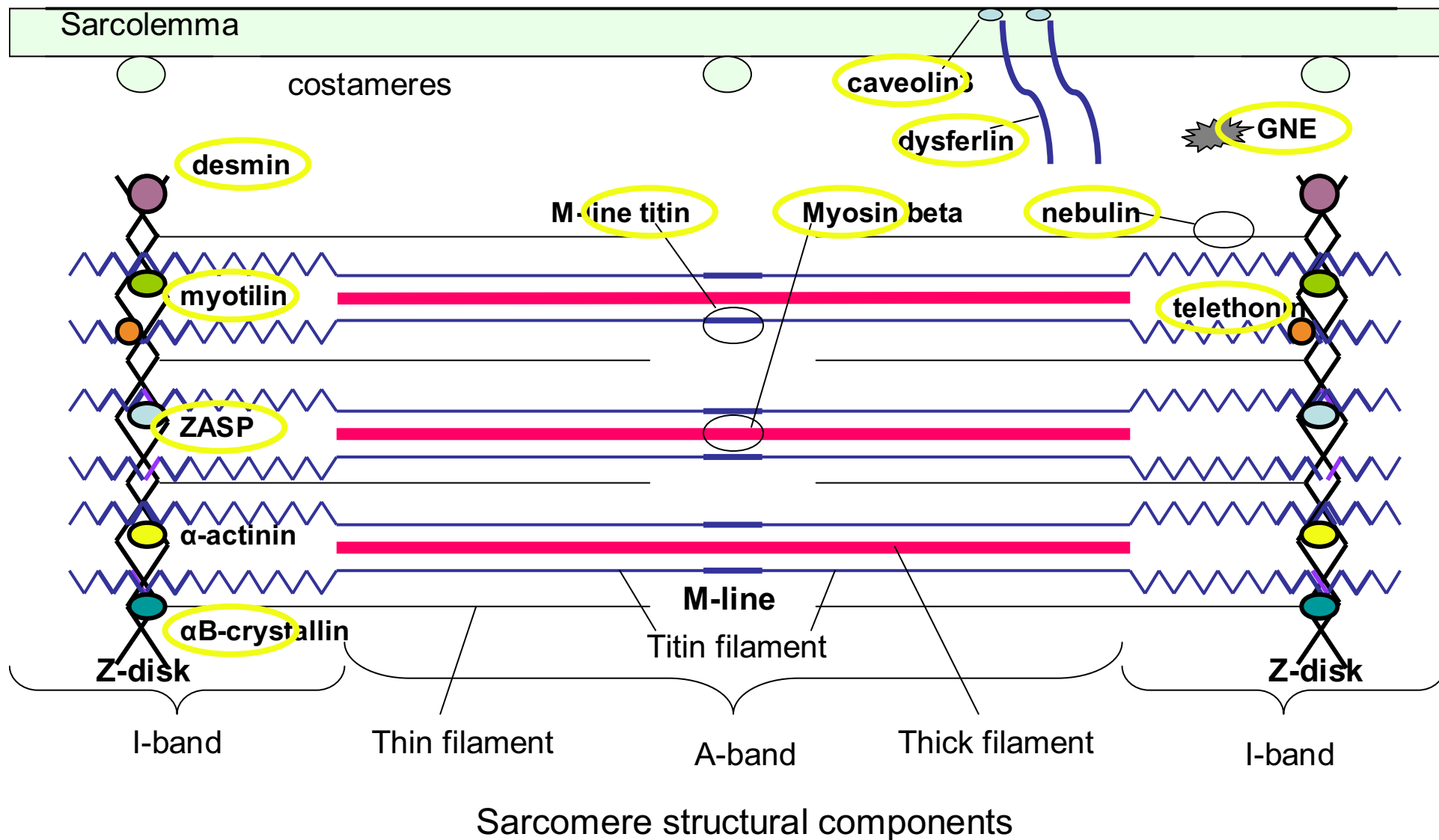
Table 1 DISTAL MYOPATHIES with genetic definition

Type	OMIM #	Onset Age	Initial weakness	CK	Muscle pathology	Fam	Gene & Locus
Laing early onset distal myopathy MPD1	#160500	1-25	Anterior lower leg	1-8x	Type 1 fiber atrophy in TA muscle, (no) vacuoles	AD	Myosin MYH7, 14q
Distal nebulin myopathy DNM	Udd pers communic	2-15	Anterior lower leg	1-3x	No nemaline rods on light microscopy, no vacuoles	AR	Nebulin NEB, 2q21
Udd distal myopathy TMD	#600334	>35	Anterior lower leg	1-4x	Dystrophic, rimmed vacuoles	AD	Titin TTN, 2q31
Distal onset in telethoninopathy	Vainzof pers comm	early	Lower leg, anterior	3-10x	Rimmed vacuoles	AR	Telethonin 17q
Desminopathy MFM (myofibrillar myopathy)	#601419	15-40	Distal leg and forearm +cardiomyopathy	1-3x	Dystrophic, rimmed vacuoles + desmin bodies	AD	Desmin 2q35
Distal myotilinopathy MFM (myofibrillar myopathy)	#609200 Penisson-Besnier	40-60	Lower legs and hands	1-3x	Rimmed and non-rimmed vacuoles, GTC dark and hyaline structures, desmin+	AD and sporadic	Myotilin 5q31
Zaspopathy MFM (myofibrillar myopathy)	#609452 Markesbery 1974	40-60	Lower legs and hands	1-3x	Rimmed and non-rimmed vacuoles, GTC dark and hyaline structures, desmin+	AD and sporadic	ZASP 10q
Distal MFM (myofibrillar myopathy)	#608810	adult	Distal leg and hands +cardiomyopathy		+desmin granulofilamentous	AD	α B-crystallin 11q
Distal Caveolinopathy	#601253	early	hands	3-10x	Reduced caveolin-3	AD	CAV3
Miyoshi myopathy MM	#254130	15-30	Posterior lower leg, calf	10-100x	Dystrophic	AR	Dysferlin 2p13
Nonaka distal myopathy DMRV/ hIBM	#605820	15-30	Anterior lower leg	1-5x	Rimmed vacuoles	AR	GNE 9p1-q1
Welander distal myopathy WDM	#604454	>40	Hands, finger extensors	1-4x	Dystrophic, rimmed vacuoles	AD	2p13
Distal myopathy with vocal cord and pharyngeal signs MPD2	#606070	35-60	Asymmetric lower leg and hands + dysphonia	1-8x	Rimmed vacuoles	AD	5q31
Distal myopathy with pes cavus and areflexia	#601846	15-50	Anterior+posterior lower leg, dysphonia+dysphagia	2-6x	Dystrophic, rimmed vacuoles	AD	19p13

Juvenile-adult onset distal myopathy	Williams	10-40	posterior – lateral lower leg	1-2x	Myopathic - dystrophic	AD	12 genetic loci excluded 8p-q and 12q linked Some 10q
Adult onset distal myopathy MPD3	Mahjneh	>30	Hands or anterior lower leg	1-4x	Dystrophic, rimmed vacuoles, + eosinophilic inclusions	AD	
MM-like non-dysferlinopathy	Linssen / Katz et al.	25-50	Posterior lower leg, calf	1-10x	Myopathic - dystrophic	AR and sporadic	

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Proteins involved in distal muscular dystrophies



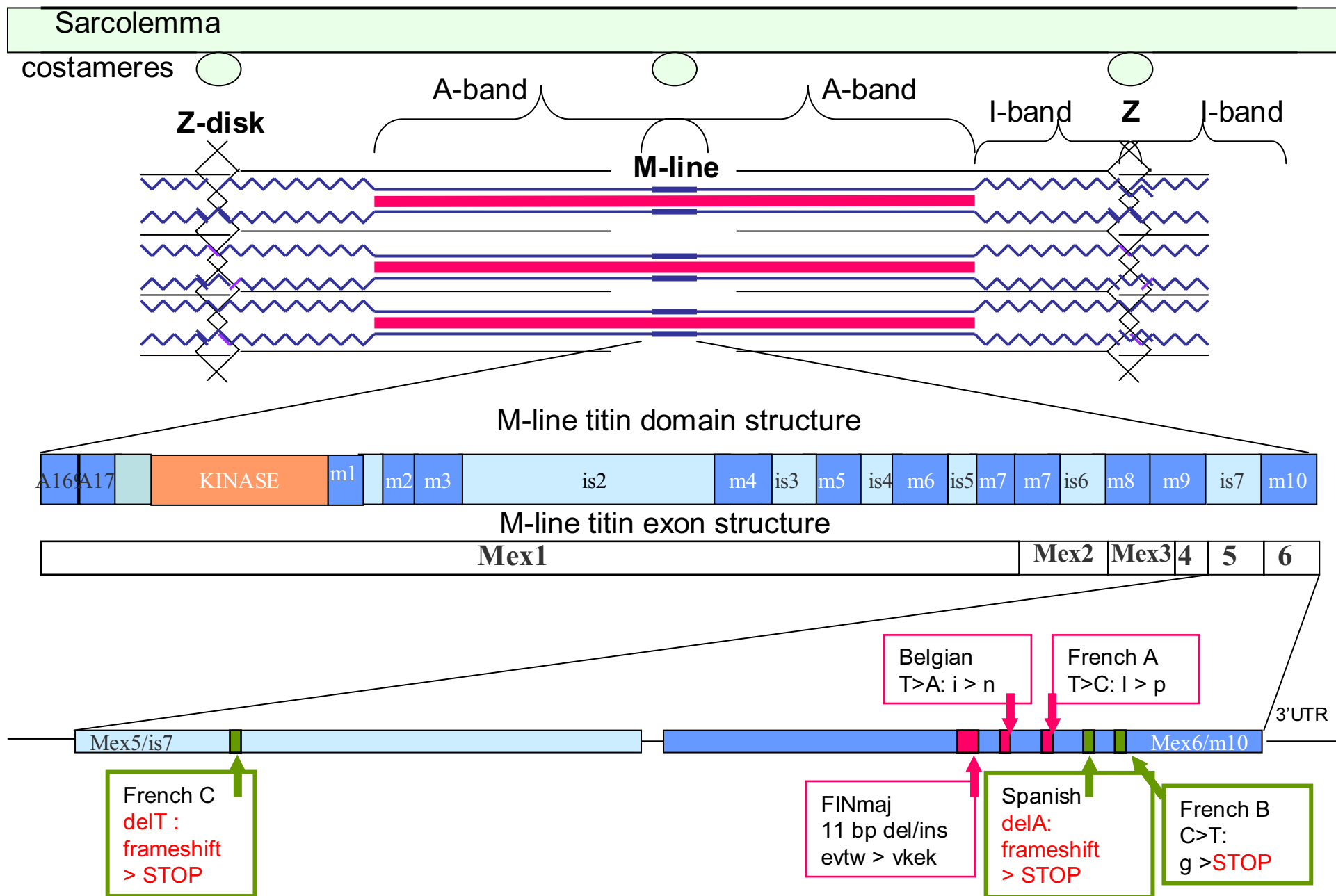


Figure 2a

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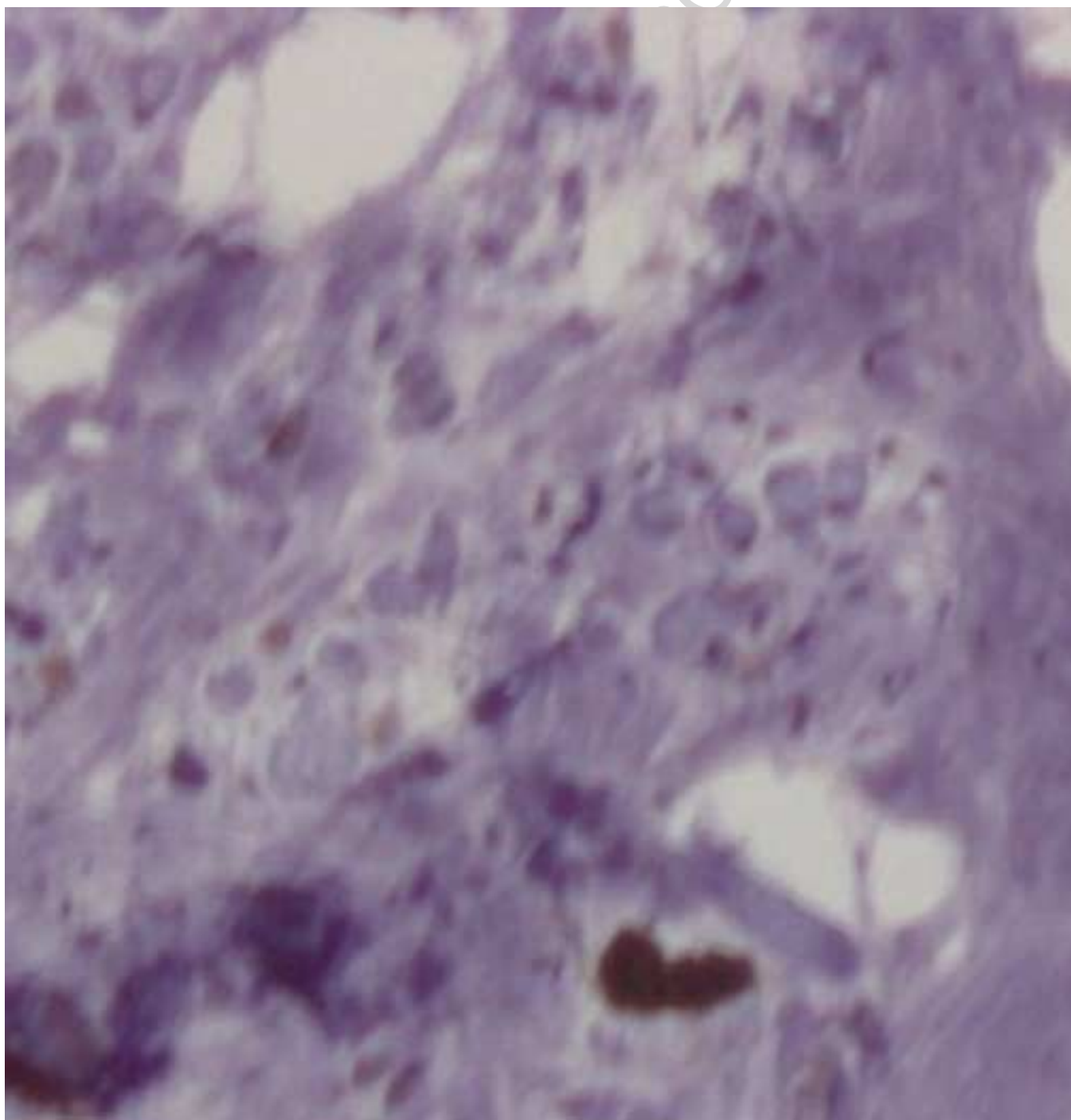
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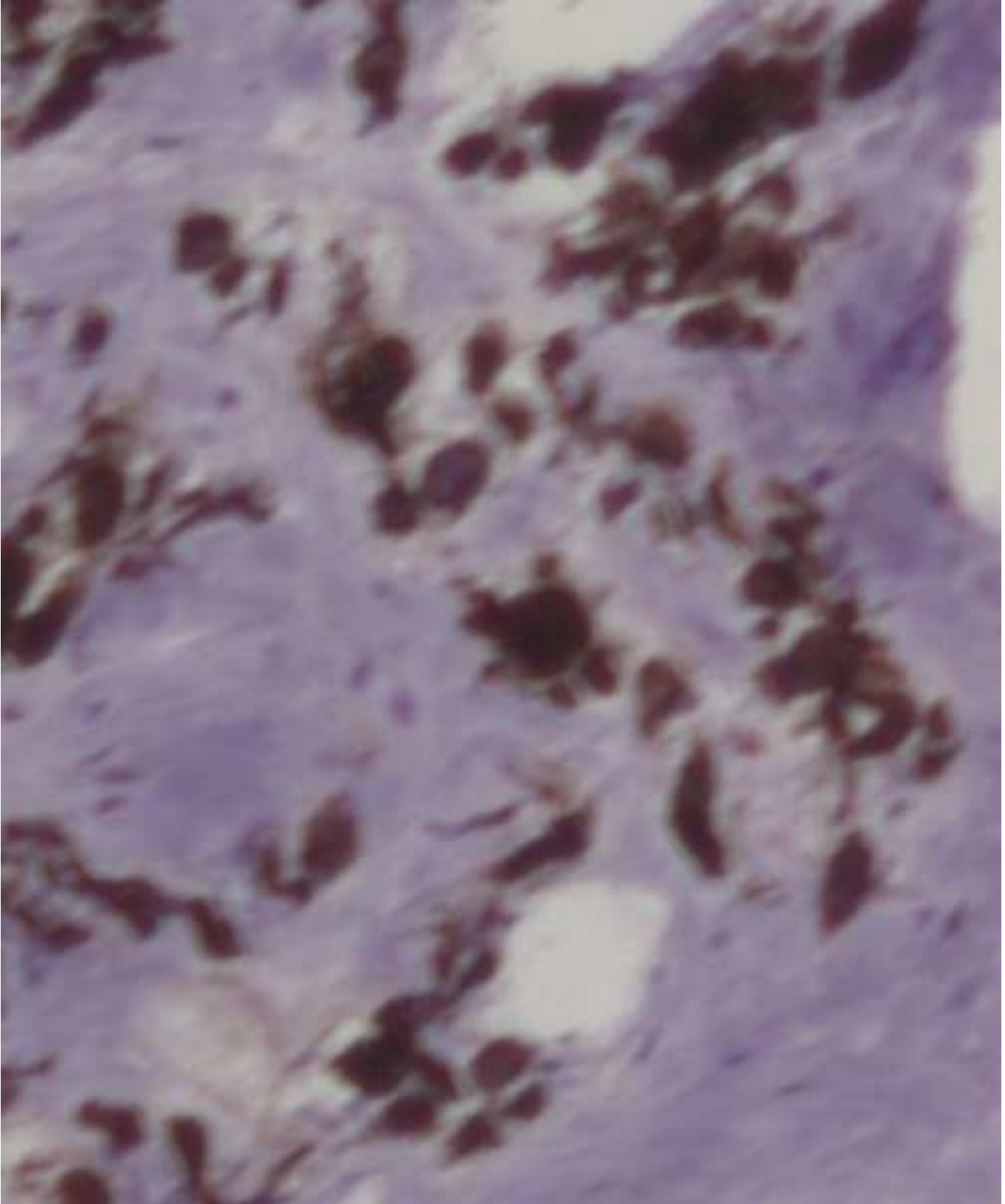


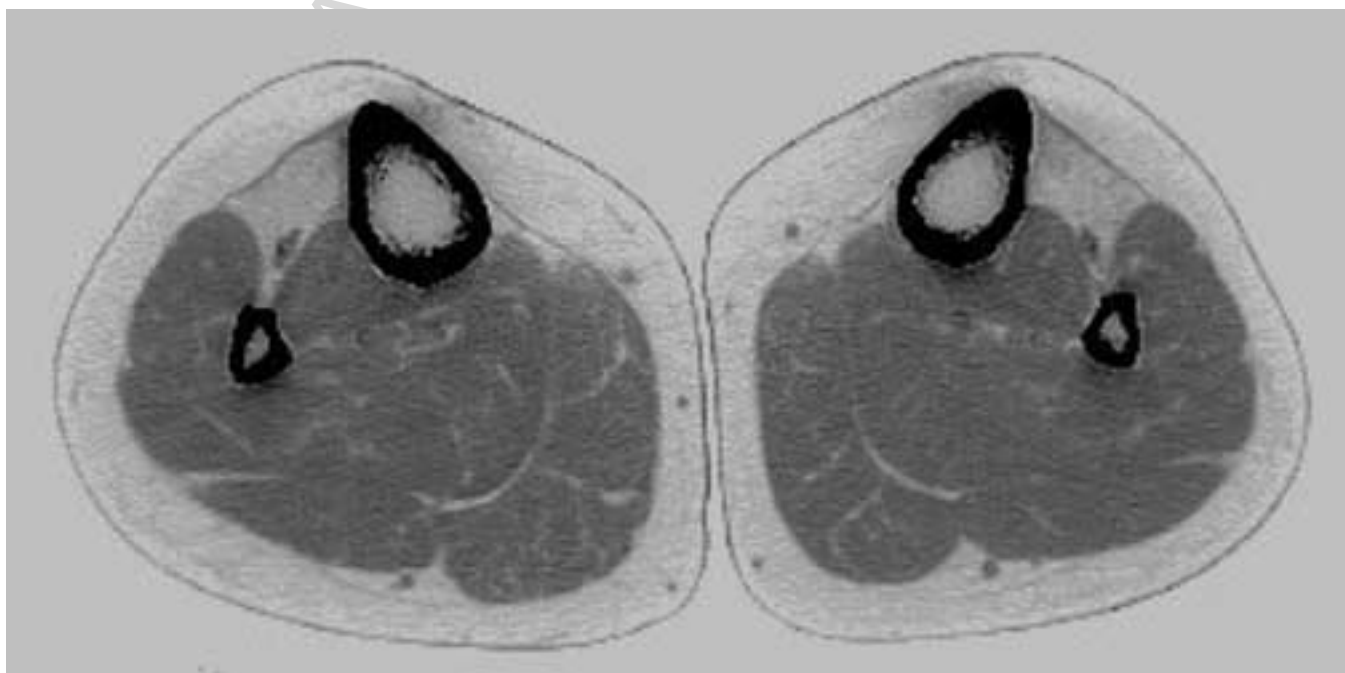


Figure 3b

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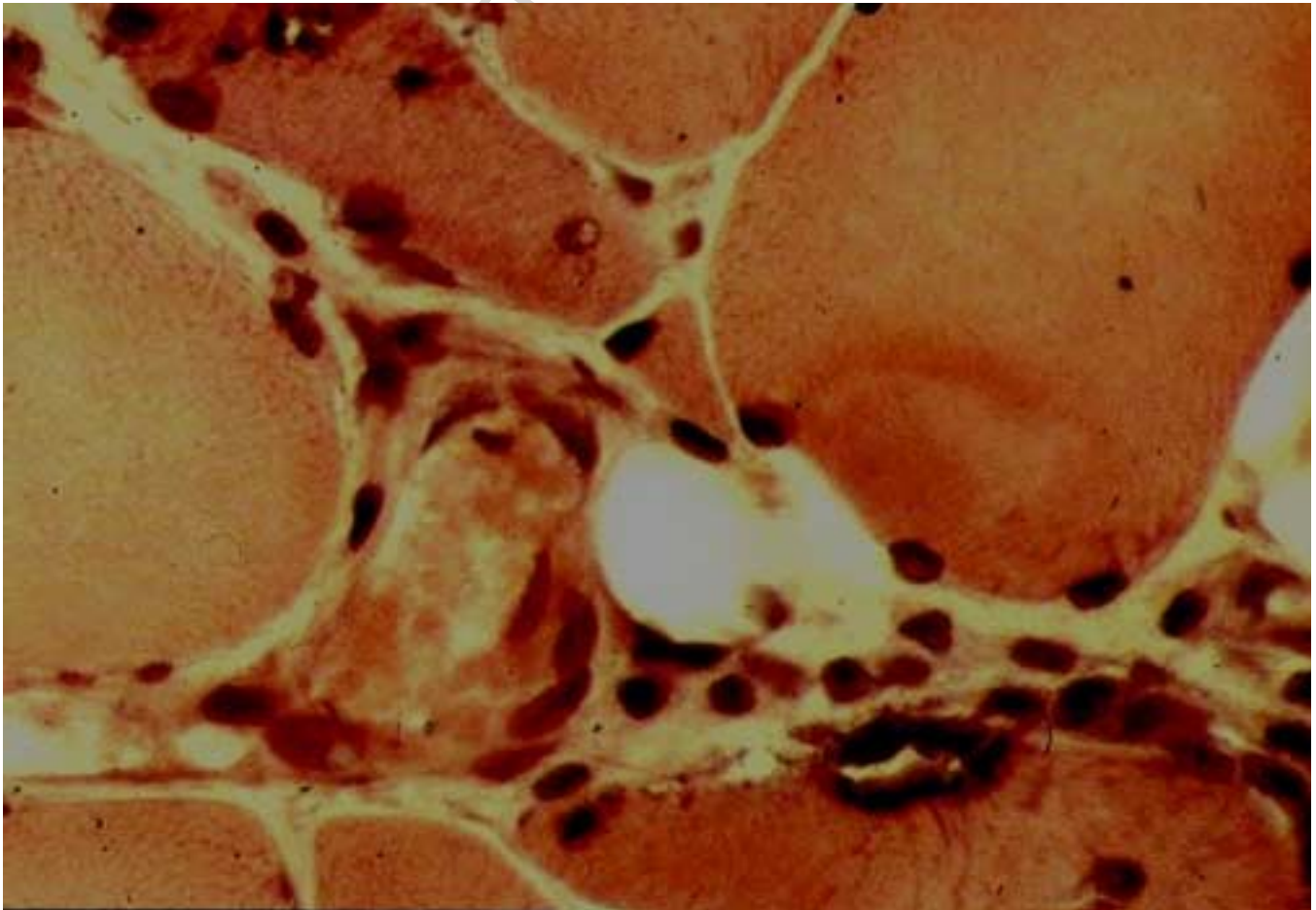


Figure 4a

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