Myofibrillar myopathy: clinical, morphological and genetic studies in 63 patients

Duygu Selcen, Kinji Ohno and Andrew G. Engel

Department of Neurology and Neuromuscular Research Laboratory, Mayo Clinic, Rochester, MN, USA

Correspondence to: Dr Duygu Selcen, Department of Neurology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA
E-mail: Selcen.Duygu@mayo.edu

Summary

The term myofibrillar myopathy (MFM) was proposed in 1996 as a non-committal term for a pathological pattern of myofibrillar dissolution associated with accumulation of myofibrillar degradation products and ectopic expression of multiple proteins that include desmin, αB-crystallin (αBC), dystrophin and congophilic amyloid material. Subsequent studies revealed dominant mutations in desmin and αBC in some MFM patients, and clinical differences between kinships. We here review the clinical, structural and genetic features of 63 unrelated patients diagnosed as having MFM at the Mayo Clinic between 1977 and 2003. The age of onset was 54 ± 16 years (mean ± SD). Weakness was both proximal and distal in 77% and proximal only in 13%. Cardiomyopathy was diagnosed in 16%. Electromyography revealed a myopathic pattern associated with abnormal electrical irritability; 13 patients had abnormal nerve conduction studies but four of these had long-standing diabetes. The abnormal muscle fibres are best identified in trichrome-stained sections as harbouring amorphous, granular or pleomorphic hyaline structures, and vacuoles containing membranous material. The hyaline structures are strongly congophilic. Semiquantitative analysis in each case indicates that among the abnormal fibres, an average of 90, 75, 75, 70 and 70% abnormally express myotilin, desmin, αBC, dystrophin and β-amyloid precursor protein, respectively. Therefore, immunostains for these proteins, and especially for myotilin, are useful adjuncts in the diagnosis of MFM. Electron microscopy shows progressive myofibrillar degeneration commencing at the Z-disk, accumulation of degraded filamentous material and entrapment of dislocated membranous organelles in autophagic vacuoles. In all patients, we searched for mutations in desmin and αBC, as well as in telethonin, a Z-disk-associated protein, or in syncoilin, which together with plectin links desmin to the Z-disk. Two of the 63 patients carry truncation mutations in the C-terminal domain of αBC, four carry missense mutations in the head or tail region of desmin, and none carries a mutation in syncoilin or telethonin. Thus, MFM is morphologically distinct but genetically heterogeneous. Further advances in defining the molecular causes of MFM will probably come from linkage studies of informative kinships or from systematic search for mutations in proteins participating in the intricate network supporting the Z-disk.

Keywords: myofibrillar myopathy; Z-disk; desmin; αB-crystallin; mutation analysis

Abbreviations: αBC = αB-crystallin; Aβ = β-amyloid protein; βAPP = β-amyloid precursor protein; CDC = cell division cycle; KPI = Kunitz protease inhibitor; LGMD = limb-girdle muscular dystrophy; MFM = myofibrillar myopathy; MUP = motor unit potential; NCAM = neural cell adhesion molecule.


Introduction

The light microscopic features of myofibrillar myopathy (MFM) were described in the 1970s and 1980s under such names as ‘myopathy with inclusion bodies’ (Nakashima et al., 1970), ‘atypical myopathy with myofibrillar aggregates’ (Kinoshita et al., 1975), ‘autosomal dominant cardiomyopathy with inclusions’ (Clark et al., 1978), ‘cardioskeletal myopathy with intrasarcoplasmic dense granulofilamentous material’ (Fardeau et al., 1978), ‘autosomal dominant spheroid body myopathy’ (Goebel et al., 1978), ‘myopathy with sarcoplasmic bodies and desmin filaments’ (Edström et al., 1978).
Table 1 Primary antibodies

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Immunogen</th>
<th>Clone</th>
<th>Source</th>
<th>Dilution/concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myotilin</td>
<td>C-terminal epitope of human myotilin</td>
<td>RS034</td>
<td>Novostra</td>
<td>1/100</td>
</tr>
<tr>
<td>Desmin</td>
<td>Whole human desmin</td>
<td>D33</td>
<td>Dako</td>
<td>0.6 μg/ml</td>
</tr>
<tr>
<td>Plectin</td>
<td>Plectin isolated from hemidesmosomes</td>
<td>HD121</td>
<td>K. Owaribe</td>
<td>1/5</td>
</tr>
<tr>
<td>αBC</td>
<td>Bovine eye lens</td>
<td>1B6.1-3G4</td>
<td>StressGen</td>
<td>0.4 μg/ml</td>
</tr>
<tr>
<td>Dystrophin</td>
<td>17 C-terminal residues</td>
<td>17</td>
<td>Santa Cruz</td>
<td>4 μg/ml</td>
</tr>
<tr>
<td>NCAM</td>
<td>Epitope 1 of all isofoms</td>
<td>17</td>
<td>Callag</td>
<td>2 μg/ml</td>
</tr>
<tr>
<td>CDC2 kinase</td>
<td>Amino acids 224–230</td>
<td>17</td>
<td>Santa Cruz</td>
<td>4 μg/ml</td>
</tr>
<tr>
<td>Gelsolin</td>
<td>Human gelsolin</td>
<td>2</td>
<td>Transduction Lab</td>
<td>5 μg/ml</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>Bovine red blood cells</td>
<td>T12</td>
<td>Chemicon</td>
<td>1/50</td>
</tr>
<tr>
<td>Titin</td>
<td>Chicken breast muscle</td>
<td>T12</td>
<td>Boehringer</td>
<td>0.5 μg/ml</td>
</tr>
<tr>
<td>βAPP N-terminal</td>
<td>N-terminal amino acids 66-81 of the pre-A4</td>
<td>22C11</td>
<td>Chemicon</td>
<td>20 μg/ml</td>
</tr>
<tr>
<td>βAPP C-terminal</td>
<td>AAP695, 21 amino acids</td>
<td>3E9</td>
<td>StressGen</td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>APP KPI domain*</td>
<td>Human APP701 amino acids 301–315</td>
<td>3E9</td>
<td>StressGen</td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>Aβ1–42*</td>
<td>C-terminal six amino acids from human Aβ 1–42</td>
<td>8E6</td>
<td>Biodesign Int</td>
<td>2 μg/ml</td>
</tr>
<tr>
<td>α1-Antichymotrypsin</td>
<td>Residues 109–112</td>
<td>3F4</td>
<td>Signet</td>
<td>8.3 μg/ml</td>
</tr>
</tbody>
</table>

*Primary antibody is mouse monoclonal unless * indicates that it is a rabbit polyclonal antibody.

1980), ‘congenital myopathy with cytoplasmic bodies’ (Wolburg et al., 1982), ‘congenital myopathy with Mallory body-like inclusions’ (Fidzianska et al., 1983) and ‘familial cardiomyopathy with subsarcolemmal vermiform deposits’ (Calderon et al., 1987). Later, however, it became apparent that some authors described the same pathological reaction under different names; that more than one pathological alteration thought to be specific for a single disorder could be present in the same muscle specimen, or even in the same muscle fibre; and that in each instance the pathological changes involve the Z-disks of the myofibril. In 1980, Edström et al. (1980) noted that some inclusions or material around them reacted for desmin. This generated the names of ‘desmin storage myopathy’ (Horowitz and Schmalbruch, 1994) and later ‘desmin-related myopathies’ (Goebel, 1997). In 1996 and 1997, detailed immunocytochemical studies revealed that many proteins, not just desmin, accumulate in the abnormal fibres, and prompted the use of the non-committal term MFM (De Bleecker et al., 1996a; Nakano et al., 1997).

Patients and methods

Patients

Sixty-three unrelated patients were diagnosed as having MFM at the Mayo Clinic between 1977 and 2003. Histochemical and genetic studies were performed in muscle biopsy specimens of all patients. All studies were in accord with the guidelines of the Mayo Institutional Review Board.

Histochemistry

Flash-frozen muscle specimens were stored in liquid nitrogen until use. For conventional histochemical studies, 10 μm frozen sections were processed for haematoxylin–eosin (H&E), modified Gomori trichrome, NADH dehydrogenase, succinate dehydrogenase, cytochrome c oxidase, adenosine triphosphatase (pre-incubation at pH 4.3, 4.6 and 9.4), acid phosphatase, periodic acid–Schiff (PAS), oil red O and non-specific esterase. Amyloid deposits were visualized with the Congo red stain and rhodamine optics (Engel, 1994).

Immunocytochemical studies

Cryostat sections 6–10 μm thick were immunostained with the primary antibodies listed in Table 1. Consecutive sections in the series were stained with trichrome. An estimate of the proportion of abnormal fibres immunoreacting for a given antigen was obtained by counting all fibres harbouring...
abnormal deposits in four randomly selected low-power fields in a trichrome-stained section and then examining the corresponding fields in an immediately adjacent immunostained section. Non-specific binding was blocked by pre-incubation with phosphate-buffered saline, pH 7.4, containing 2% bovine serum albumin and 10% donkey or goat serum. Myotilin, desmin, αBC, dystrophin, plectin, cell division cycle (CDC) 2 kinase, prion protein, neural cell adhesion molecule (NCAM), gelsolin, ubiquitin, the N- and C-terminal and Kunitz protease inhibitor (KPI) domains of β-amyloid precursor protein (βAPP) and β-amyloid (Aβ) protein were localized with the immunoperoxidase method using a biotinylated secondary antibody and the ABC kit (Vector Laboratories, Burlingame, CA). α-Actinin, nebulin and titin were visualized by immunofluorescence, with detection of the first antibody with Cy3-labelled donkey anti-mouse IgG, or with biotinylated goat anti-mouse IgG followed by fluorescein isothiocyanate- or Cy3-labelled streptavidin. Filamentous actin was stained with rhodamine–phalloidin, or with biotinylated goat anti-mouse IgG followed by fluorescein isothiocyanate- or Cy3-labelled streptavidin. Controls consisted of replacement of monoclonal antibodies with non-immune IgG of the same subclass and concentration as the primary antibody, and replacement of polyclonal antibodies by pooled non-immune IgG.

Electron microscopy
Muscle specimens were fixed at rest length and processed for electron microscopy by standard methods (Engel, 1994).

Molecular genetic studies
DNA was isolated from muscle using the phenol/chloroform method (Sambrook and Russell, 2001). mRNA was obtained using the Micro-FastTrack mRNA isolation kit (Invitrogen). First strand cDNA was prepared from mRNA by using random hexamer primers with the cDNA cycle kit (Invitrogen). Polymerase chain reaction (PCR) primers were designed to analyse all exons and their flanking non-coding regions of desmin (DES), αBC (CRYAB), telethonin (TCAP) and syncoilin (SYNC) using published genomic sequences obtained from the human genome project. Published sequences of DES and CRYAB mRNA were used to design cDNA primers. PCR-amplified fragments were purified using the QIAquick 8 PCR Purification Kit (Qiagen), and directly sequenced with an ABI377 DNA sequencer (Applied Biosystems) using fluorescence-labelled dideoxy terminators. After identifying mutations in DES or CRYAB, allele-specific PCR was employed to screen for the identified mutations in 200 normal alleles of 100 unrelated control subjects. The entire coding region of DES was amplified from normal human muscle mRNA using nested reverse transcription (RT)–PCR. The nested forward primer introduced the Kozak consensus sequence (5’-CCACC-3’) before the translational start site (Kozak, 1987). The nested forward

and reverse primers also carried EcoRI and HindIII recognition sites, respectively, at their 5’ ends. The PCR product was then ligated into pEGFP-N1, a plasmid harbouring the gene encoding enhanced green fluorescent protein (EGFP; Clontech), to attach EGFP to the C-terminal end of desmin. The mutated human desmin cDNA clones were obtained by introduction of the mutated oligonucleotides into pEGFP-DES using the Quik-Change Site Direct Mutagenesis kit (Stratagene). For each construct, the presence of the mutation and absence of unwanted artefacts were confirmed by sequencing of the entire insert. Wild-type and mutant pEGFP-DES constructs were transfected into MCF7 cells derived from human breast adenocarcinoma cells, and into COS cells derived from green monkey kidney fibroblasts. Cells were transfected 24 h after plating using Fugene 6 (Roche) according to the manufacturer’s instructions. At 48 h post-transfection, the cells were fixed and analysed with a Zeiss epifluorescence microscope. Each transfection was repeated three times.

Results
Clinical characteristics of patients
Twenty-nine patients were women and 34 were men. The age of onset varied from 7 to 77 years (mean, 54 years; median 55 years) but only one patient presented before the age of 10 years, and only three before the age of 20 years. The age at diagnosis ranged from 21 to 82 years (mean, 62 years, median 64 years). Sixteen patients had evidence for autosomal dominant inheritance. Five patients had similarly affected family members, siblings or cousins, but the pattern of inheritance could not be determined with confidence. Symptoms elicited at the time of diagnosis consisted of slowly progressive weakness, 58; paresthesias, 10; muscle wasting, nine; muscle stiffness, aching or cramps, eight; dyspnoea, eight; dysphagia, four; head drop due to isolated neck extensor weakness, one; and palpitation, one.

Examination at the time of diagnosis revealed muscle weakness in all patients. The distribution of weakness was determined in 56 patients. In 41, the weakness involved both proximal and distal muscles; in 16 the weakness was equal proximally and distally; and in 16 it was greater distally than proximally. In 13 patients, the weakness was only proximal, and in two it was only distal. In seven patients, the weakness was asymmetric in distribution. Four patients had facial as well as limb weakness. None of the patients presented with a scapuloperoneal pattern of weakness. Ten patients had conspicuous muscle atrophy. One patient had elbow, wrist and hip contractures. Two patients had profound distal sensory deficits.

Ten patients had cardiomyopathy at the time of diagnosis. Two of these patients had signs of congestive heart failure and arrhythmia; three had signs of congestive heart failure without arrhythmia; four had arrhythmia without congestive failure; and one patient had pre-symptomatic dilated
cardiomyopathy discovered when the muscle biopsy findings of MFM prompted an echocardiographic examination. Two of the 10 patients with cardiomyopathy had a dominantly inherited mutation in desmin. Ten other patients had miscellaneous electrocardiographic abnormalities compatible with cardiomyopathy (conduction block, prolonged QT interval, intermittent atrial fibrillation, unusual depolarization abnormality and premature ventricular complexes). Two of these patients had a mutation in desmin; in one case, the mutation was sporadic, in the other it was dominantly inherited. None of the 20 patients with cardiac abnormalities had mutations in αBC.

The serum creatine kinase (CK) level was normal in 23 patients, <7-fold above the upper normal limit in 30, and not determined in 10.

EMG studies were obtained in 57 patients. Myopathic motor unit potentials (MUPs) were recorded in 31, and both myogenic and high-amplitude long-duration MUPs in 19.

![Fig. 1](image-url) Histochemical and immunocytochemical features of myofibrillar myopathy. Note accumulation of desmin (B), αBC (C), dystrophin (D), CDC2 kinase (F), βAPP (G), prion protein (H), NCAM (J), and myotilin (L), and sharply circumscribed decreases of oxidative enzyme activity (K) in fibre regions that appear abnormal in serial trichrome-stained sections (A, E and I). Intensely fluorescent congophilic deposits (M and N). Bar in A (also applies to B–L) = 40 μm; bars in M and N = 20 μm.
Abnormal electrical irritability, consisting of fibrillation potentials, positive sharp waves and complex repetitive discharges, was present in 30 patients; 15 of these also had myotonic discharges. In two patients, abnormal electrical irritability was the sole EMG abnormality. Thirteen patients had abnormal nerve conduction studies consistent with polyneuropathy, but four of these patients had long-standing diabetes as well.

**Histological features**

In all cases, the muscle fibres varied abnormally in size, with some fibre diameters as small as 5 μm or as large as 150 μm. Some abnormal fibres contained large vesicular nuclei. Seventy percent of the muscle specimens contained groups of small fibres, with three or more small fibres per group. In most cases, however, the atrophic fibres accounted for only a small proportion of the total. In each case, some fibres were subdividing by splitting, and some small fibres arose by this mechanism. Sparse perivascular or endomysial mononuclear inflammatory cells appeared in <10% of the biopsy specimens. Necrotic fibres were uncommon. Regenerating fibres, identified by cytoplasmic basophilia and vesicular nuclei with prominent nucleoli, were infrequent.

In all cases, the structurally abnormal muscle fibres were best observed in trichrome-stained sections. Typically, these fibres were unevenly distributed, few or none appearing in some fascicles and many present in others. The abnormal fibres displayed one or more of the following features.

(i) Abnormal areas containing blue amorphous material. In a given fibre, these areas were single or multiple, varied in shape and size, were superficial or deep in position, and encompassed from a small fraction to nearly the entire extent of the cross-sectioned fibre (Fig. 1A, E and I).

(ii) Abnormal fibre areas containing small (<6 μm) blue or blue–green granules. These areas varied in shape, size and position in the affected fibres (Fig. 1E and I).

(iii) Spherical, lobulated or serpentine hyaline structures (Fig. 1E and I), blue to purple in colour and sometimes with a central red zone; these varied in size, position and abundance in the abnormal fibres.

(iv) Typical cytoplasmic bodies were conspicuous in <10% of the specimens; a smaller proportion of the abnormal fibres harboured nemaline rods.

(v) Some abnormal fibres harboured small vacuoles rimmed or filled by membranous material. In four patients, the vacuolar change was prominent.

(vi) Some large fibres were completely filled with amorphous, granular or hyaline material (Fig. 1E).

In most specimens, the hyaline structures were congophilic. The congophilia was best observed in Congo red-stained sections viewed under rhodamine optics (Fig. 1M and N). The intensity of the fluorescent signal varied from mild to very intense in the same and different specimens.

Oxidative enzyme activity was sharply reduced in fibre regions occupied by the hyaline structures and in many other abnormal fibre regions (Fig. 1K). Increases of acid phosphatase were detected in some vacuoles and in small foci of many abnormal fibres. The atrophic fibres appearing in groups were of either histochemical type. Grouping of the histochemical fibre types was present in 25%, and type 1 fibre predominance in 15% of the specimens. In one-half of the cases with fibre type grouping, the EMG revealed high-amplitude long-duration MUPs. Type 2 fibre atrophy was uncommon. Many abnormal fibres contained small lakes of PAS-positive material. The muscle fibre lipid content was normal.

**Immunocytochemical studies**

### Myotilin

We were interested in examining expression of myotilin in MFM because it is a recently discovered Z-disk-associated protein that cross-links actin filaments, controls sarcomere assembly (Salmikangas et al., 2003) and binds to α-actinin and γ-filamin (van der Ven et al., 2000). Increased myotilin expression was detected in ~90% of the fibres that were abnormal in trichrome-stained sections (Fig. 1L).

### Desmin

In all cases, desmin expression was moderately to markedly enhanced in >75% of the abnormal fibres (Fig. 1B). The desmin deposits were associated with amorphous and granular material and often surrounded or appeared within the hyaline structures.

### α-β-γ

The α-β-γ deposits resembled the desmin deposits in frequency, distribution and staining intensity (Fig. 1C).

### Dystrophin

Ectopic cytoplasmic expression of dystrophin was present in close to 70% of the abnormal fibres (Fig. 1D).

### β-APP and Aβ

Increased expression of both N- and C-terminal epitopes of β-APP was observed in granular, hyaline as well as amorphous fibre regions in close to 70% of the abnormal fibres (Fig. 1G). In contrast, only faint expression of Aβ42 and of the KPI domain of β-APP was detected in some fibres in a few specimens. There was no consistent correlation between the increased expression of β-APP and the presence of the congophilic deposits in the fibres.

### NCAM

Enhanced NCAM expression was observed in 50–60% of the structurally abnormal fibres (Fig. 1J), in regenerating and
some atrophic fibres, and also in some structurally normal fibres.

Filamentous actin
When monitored with rhodamine-labelled phalloidin, ~50% of the abnormal fibres showed foci of enhanced actin expression. Occasional fibres showed circumscribed decreases of actin expression.

CDC kinase 2
CDC2 kinase (a mitotic kinase that phosphorylates and disassembles intermediate filaments) was expressed in ~40% of the abnormal fibres, and sometimes in otherwise normal fibres (Fig. 1F).

Prion protein
Prion protein expression was enhanced in 60% of the abnormal fibres (Fig. 1H).

Plectin
Punctate or diffuse increases of plectin were observed in non-hyaline regions of 50–60% of the abnormal fibres; the increase was mild in most fibres and strong in a few. The hyaline structures, however, were consistently devoid of plectin.

Other proteins
Enhanced expression of α1-antichymotrypsin, gelsolin and ubiquitin was found in about one-third of the abnormal fibres. In most specimens, the increase in expression of these proteins was only mild, but in a few specimens it was striking.

Ultrastructural features
In each case, the earliest ultrastructural abnormality involved the myofibril and characteristically the Z-disk. In some fibre regions, normal Z-disks gave rise to and were replaced by large irregular expanses of homogeneous (Fig. 2A) or striped (Fig. 2B) dense material, or small dappled dense bodies of Z-disk intensity (Fig. 2A and C). Some abnormal fibre regions also showed Z-disk streaming as well as accumulation of dappled dense bodies and myeloid structures between the myofibrils (Fig. 2C and D).

In more advanced lesions, large fibre areas were replaced by inclusions composed of abnormal dense material intermingled with dappled dense bodies (Fig. 3A), or dense fibrillar material (Fig. 3B). Both types of inclusions were surrounded by a halo of fine filaments. In other fibre regions, small electron-dense spots were intermingled with randomly oriented fine filaments (Fig. 3C and D). Still other abnormal fibre regions were composed only of a filamentous network (Fig. 4A and B). In some cases, the degraded myofibrillar material accumulated in large spheroidal structures. Typical cytoplasmic bodies surrounded by a halo of radially arranged thin filaments were infrequent. All these abnormal areas were devoid of mitochondria. Similar ultrastructural findings were detected in patients with or without mutations in desmin or αBC.

In many fibres, small islands of myofibrils were surrounded by degraded filamentous material, degenerating membranous organelles and myeloid structures (Fig. 5). Mitochondria, other membranous organelles and glycogen accumulated in spaces vacated by myofibrils (Figs 5 and 6A). The dislocated membranous organelles were trapped and degraded to myeloid structures in autophagic vacuoles (Fig. 6B).

Molecular genetic studies
DNA obtained from all 63 patients was analysed for mutations in desmin, αBC, syncoilin and telethonin. Four patients carried novel missense mutations in desmin. Three of these mutations were located in the desmin head domain (S2I, S46F and S46Y) and one in the desmin tail domain (K449T) (Fig. 7). The mutated residues, S2, S46 and K449, are conserved across mammalian species and in Xenopus.

Transfection of COS and MCF7 cells with the wild-type pEGFP-desmin construct yielded three expression patterns in both types of cells: diffuse expression in a few cells, lace-like filaments in some cells, and coarse, thick and short filaments in other cells. Entirely similar expression patterns were observed after transfection of COS and MCF7 cells with mutant pEGFP-desmin constructs. Thus, these expression systems yielded no insight into the pathogenic effects of the desmin mutants in vivo.

Two MFM patients carried truncation mutations in the C-terminal domain of αBC (464delCT and Q151X). Further characterization of these mutations was published separately (Selcen and Engel, 2003). None of the mutations was detected in 200 alleles of 100 unrelated control subjects.

None of the MFM patients harboured mutations in telethonin or syncoilin.

Differences in MFM with or without identified mutations in desmin or αBC
The findings in muscle specimens with or without mutations in desmin or αBC were generally similar, with the following exceptions. With mutations in desmin or αBC (i) the abnormal fibre regions were often smaller and less conspicuous; (ii) the congophilic deposits were less numerous and less intensely fluorescent; (iii) ubiquitin expression was not enhanced; and (iv) myotilin expression was less intense and ectopic dystrophin expression more intense than in the remaining MFM patients. However, none of these morphological differences provided a basis for confidently identifying patients with mutations in desmin or αBC.
Discussion

The morphological features of MFM differentiate it from other chronic myopathies. Although these features implicate myofibril- and especially Z-disk-associated proteins, they do not specify the molecular aetiology. Only six of the 63 patients in the present series carried mutations in desmin or αBC. Thus, the molecular basis of 90% of MFM patients in our cohort remains undetermined.

The typical light microscopy features pointing to the diagnosis of MFM are (i) abnormal fibre regions of varying size and shape, harbouring amorphous, granular or hyaline structures in trichrome-stained sections; (ii) sharply circumscribed decreases of oxidative enzyme activity in many abnormal fibre regions; (iii) prominent congophilic deposits associated with hyaline structures; (iv) vacuolated muscle fibres with membranous material within or lining the vacuoles; (v) frequent neurogenic features, and especially small groups of atrophic fibres of either histochemical type; and (vi) abnormal and ectopic expression of multiple proteins. Not all ectopically expressed proteins appear in all

Fig. 2 (A) Cones of dense material and dappled dense bodies emanate from and replace normal Z-disks. In (B), normal Z-disks are replaced by stripes of dense material and the myofibrils move out of register. In (C), widened intermyofibrillar spaces are filled with dappled dense bodies and some Z-disks show streaming (asterisk). In (D), the abnormal fibre region harbours streaming Z-disks, dappled dense bodies, degenerating membranous organelles and small nemaline rods (R). Z = Z-disk. Bars = 10 μm.
structurally abnormal fibres. The most commonly observed proteins are myotilin, desmin, αBC, βAPP and dystrophin. Therefore, immunostains for these proteins are especially useful adjuncts in the diagnosis of MFM. Previous studies of MFM have not examined myotilin expression; our observations suggest that it is the most reliable immunocytochemical marker for abnormal fibre regions in MFM.

Electron microscopy provides further insight into the pathogenesis of MFM and suggests the following sequence of events: (i) disintegration of myofibrils that begins at the Z-disk; (ii) loss of normal myofibrillar and then of myofibre architecture; (iii) accumulation of degraded filamentous material in various patterns; (iv) dislocation and aggregation of membranous organelles and glycogen in spaces vacated by myofibrils; and (v) entrapment of dislocated membranous organelles in autophagic vacuoles.

The increased αBC expression could be a response to disruption of the myofibrillar architecture and is consistent with the chaperone role of αBC for desmin and actin (Bennardini et al., 1992). Desmin and NCAM are markers of regenerating muscle fibres, but both of these proteins also accumulate in abnormal fibre regions (Cashman et al., 1987; Bornemann and Schmalbruch, 1992). Accumulation of actin, myotilin and desmin, as well as of plectin, an

Fig. 3 Various MFM inclusions. The inclusion in (A) consists of dense material and dappled dense bodies. Inclusions in (B) and (C) arise from striped material derived from Z-disks. (D) On higher magnification, most MFM inclusions consist of a mesh of fragmented filaments. Bars = 10 μm.
intermediate filament linker, may be compensatory to maintain Z-disk structure as all these proteins are Z-disk related and play a role in Z-disk organization (Faulkner et al., 2001; Clark et al., 2002). The accumulation of the actin-severing protein gelsolin suggests that it participates in fragmenting the accumulated actin filaments. CDC2 kinase phosphorylates and disassembles intermediate filaments during mitosis; its inappropriate expression in MFM may result in phosphorylation of desmin and destabilization of the Z-disks (Nakano et al., 1997). The reason for ectopic expression of dystrophin and prion protein, both membrane-associated proteins, is unclear. The ubiquitin positivity in some specimens indicates that the muscle fibre contains undesirable material targeted for non-lysosomal degradation. However, lack of significant ubiquitin positivity in most cases implies operation of an alternative pathway of protein degradation. The increased expression of the serine protease inhibitor α1-antichymotrypsin may be compensatory to mitigate fibre damage by proteolytic enzymes. The intense congophilia indicates a β-pleated conformation of aggregated proteins. Electron microscopy of MFM, however, does not reveal amyloid filaments of the type detected in systemic amyloidosis. Although expression of βAPP is enhanced, there is only mild or no expression of Aβ. The chemical identity of congophilic material in MFM remains unknown.

**Fig. 4** In (A) and (B), the filaments in the inclusions are of the same size or thinner than thin filaments in the remaining normal myofibrils. Bars = 5 μm.

**Fig. 5** Large abnormal fibre region harbours fragmented filaments, dislocated mitochondria and myeloid structures. Arrows point to persisting normal myofibrils. ×11 200. Bar = 10 μm.
Although there are minor differences of protein expression in MFM patients with or without identified mutations in desmin or αBC, these differences are not sufficient to identify the mutation carriers. Moreover, the increased expression of any one protein does not point to an underlying mutation in a given patient, as similarly enhanced expression of desmin and αBC occurs in patients with or without mutations in these proteins. However, one can infer that the stereotyped ectopic expression of multiple proteins in MFM is the end result of a pathogenic cascade initiated when myofibrillar integrity is disrupted by different insults. Consistent with this idea is that target formations in partially denervated muscle and unstructured cores in central core disease also contain many of the abnormally expressed proteins detected in MFM lesions (De Bleecker et al., 1996b; Nakano et al., 1997).

**Desmin mutations**

The observed mutations are in the head and tail domains of desmin, whereas most previously reported mutations are in the desmin rod domain that is crucial for desmin filament assembly (see Fig. 7). The pathogenic effects of the mutations observed by us could not be assessed when the genetically engineered mutants were expressed in COS cells or MCF7 cells. A likely reason for this is that the observed mutations do not hinder the assembly of desmin filaments and that neither...
expression systems subjects the desmin cytoskeleton to the mechanical stress of repeated contractions that occur in muscle in vivo. Consistent with our observations, the previously reported I451M mutation in the desmin tail domain also had no effect on the organization of the desmin cytoskeleton in C2.7, MCF7 and vimentin-positive or negative SW13 cells (Dalakas et al., 2003).

**Candidate genes or proteins for MFM**

The MFM genes mapped to 2q24–31 (Edström et al., 1990; Nicolao et al., 1999), 10q22.3 (Melberg et al., 1999) and 12q (Wilhelmsen et al., 1996) are still unidentified. Other candidate genes encode proteins located or anchored at the Z-disk, or play a role in maintaining myofibrillar integrity.

Actin filaments, the actin-associated nebulin filaments and the thick-filament-associated titin filaments are anchored in the Z-disk. Within the Z-disk, telethonin caps titin, and titin has the capability of phosphorylating telethonin (Labeit et al., 1997; Sorimachi et al., 1997). Telethonin also binds to myozenin (also known as FATZ or calscarin) which binds to filamin C (Faulkner et al., 2000; Takada et al., 2001; Zhou et al., 2001) which, in turn, is linked to myotilin (van der Ven et al., 2000), and myotilin itself interacts with α-actinin (Hauser et al., 2000) and actin (Salminen et al., 2003). Myopalladin tethers nebulin to α-actinin and probably plays a role in Z-disk assembly (Bang et al., 2001). Cypher also binds to α-actinin and supports the Z-disk during contraction (Zhou et al., 1999, 2001; Faulkner et al., 1999). Myopodin co-localizes with α-actinin at the Z-disk and binds actin (Weins et al., 2001). CapZ organizes actin filaments during sarcocere formation and also localizes to the Z-disk (Schafer et al., 1995). Syscofilin interacts with desmin and, together with plectin, may link desmin filaments to the Z-disk (Newey et al., 2001; Poon et al., 2002; Howman et al., 2003). An isoform of neuroendocrine-specific-protein-like-I also co-localizes with desmin and is distributed throughout the Z-disk (Geisler et al., 1999). Synefin (also known as desmuslin) is an intermediate filament that forms hybrid filaments with desmin and has a binding site for α-actinin (Bellin et al., 1999; Mizuno et al., 2001). The associated intermediate filaments form a heteropolymeric lattice to organize the myofibrils at the level of the Z-disk, and to link the myofibrils to nuclei, mitochondria and the sarclemma.

Some Z-disk-related proteins have already been implicated in inherited myopathies, e.g., mutations in actin are associated with actin myopathy and nemaline myopathy (Nowak et al., 1999); mutations in nebulin also result in nemaline myopathy (Pelin et al., 2002); telethonin mutations underlie limb-girdle muscular dystrophy (LGMD) 2G (Moreira et al., 2001), or dilated cardiomyopathy (Hackman et al., 2002), a form of LGMD (Udd et al., 1991; Haravuori et al., 2001), or dilated cardiomyopathy (Hackman et al., 2002). In the nemaline and actin myopathies, the morphological alterations are unlike those in MFM, but the possibility remains that other mutations in nebulin or actin could result in an MFM phenotype. The immunocytochemical and ultrastructural features of dystrophies stemming from mutations in titin and myotilin have not been characterized extensively, and in these disorders MFM features may have gone undetected.

Further advances in defining the molecular causes of MFM will probably come from linkage studies of informative kinships and a search for mutations at identified loci, or from systematic search for mutations in proteins participating in the intricate and interconnected network supporting the Z-disk.

**Acknowledgements**

We wish to thank Adela Graves, Mary Olson and Lois Rowe for technical assistance. This work was supported by a CR20 Grant from the Mayo Foundation to D.S.

**References**


Asijee GM, Sturk A, Bruin T, Wilkinson JM, Ten Cate JW. Vinculin is a permanent component of the membrane skeleton and is incorporated into the (re)organising cytoskeleton upon platelet activation. Eur J Biochem 1990; 189: 131–6.


