Multi-minicore myopathy: a clinical and histopathological study of 17 cases

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Abstract

Multi-minicore disease (MmD) is a congenital myopathy morphologically defined by the multifocal lack of oxidative activity on light microscopy (LM) and multiple small zones of sarcomeric disorganization on electron microscopy (EM) as the main findings in muscle biopsy.

We report on clinical and pathomorphological features of 17 patients diagnosed with multi-minicore myopathy at our department.

Clinically, axial and proximal muscle weakness was the predominant distinguishing feature. Dysmorphic features such as high-arched palate and chest deformities were frequent findings. Limitation in cervical spine mobility was found in 4 cases. Most of our cases were slowly progressive but three fatal cases also occurred.

Multifocal lack of oxidative activity was found in 16/22 biopsies on LM. Examination on EM enabled the final diagnosis of MmD in all cases. It is of special interest that in 3 patients fulfilling the criteria of pure congenital fibre type disproportion and in 2 cases of centronuclear myopathy, the findings of ultrastructural examination led us to a revised diagnosis of MmD. We postulate that all muscle biopsies with abnormal fibre proportion or centrally located nuclei as the only pathology on LM need to undergo careful EM evaluation to identify possible underlying multi-minicore disease.

Key words: muscle, pathomorphology, congenital myopathy, light microscopy, electron microscopy, fibre type disproportion.

Introduction

Minicore myopathy (multi-minicore disease; MmD) was first described by Andrew Engel et al. in 1971 [6]. This congenital myopathy can only be diagnosed by means of muscle biopsy and is morphologically characterized by the presence of multifocal lack of oxidative activity on light microscopy (LM) and multiple small zones of sarcomeric disorganizations and mitochondrial depletion on electron microscopy (EM). Minicores differ from cores (which are characteristic of the central core disease) by their smaller diameter and being limited to some sarcomeres. The minicores are present in both type 1...
and 2 fibres. In addition to basic morphological changes, fibre type disproportion was reported in most cases. Centrally located nuclei have also been reported in muscle biopsies of MmD patients [11,17]. In some cases, minicores may not be clearly visible on light microscopy [7,15].

Clinically, MmD is a slowly progressing congenital myopathy, marked by generalized weakness and muscle hypotonia. Four clinical subtypes of MmD were recently identified [7,6,15,18]. The most common „classical” phenotype of MmD is characterized by marked axial weakness, scoliosis with spinal rigidity and marked respiratory impairment. Manifestations of the moderate form include generalized muscle weakness pronounced in the pelvic girdle. Scoliosis and respiratory muscle involvement is mild or absent in this subgroup of patients. The two other forms of the disease are characterized by a clinical picture similar to the classical one, but they may also include partial or complete external ophthalmoplegia, and antenatal onset with arthrogryposis, respectively.

The formation of minicores may well be a non-specific feature. Occasional minicores have been seen in Marfan’s syndrome [23], the cerebro-retinomuscular syndrome [1], type III glycogenosis [25], short chain acyl-CoA dehydrogenase deficiency [31], inflammatory myopathies [35], denervation muscle atrophy [28] and even in healthy young subjects [21].

MmD is one of the structural congenital myopathies, the genetic basis for which is only partially known. An autosomal recessive pattern of inheritance is generally postulated, but dominant forms have also been described [24,34,36]. Recessive mutations in the SEPN1 gene, implicated in congenital muscular dystrophy with early rigidity of the spine [22], have been recently identified in >40 patients with the classical MmD phenotype [9]. In addition, the discovery of a recessive mutation in the RYR1 gene has provided the genetic basis for the known clinical overlap between the central core disease and MmD with distal muscle involvement [16]. Of special interest is a recent report by Guis et al. [14], who described a large family with malignant hyperthermia (MH) in which multi-minicores on muscle biopsy were observed in MH susceptible patients. Genetic analysis revealed that the association between multi-minicores and MH susceptibility was linked to novel mutations in the RYR1 gene [14].

The aim of this study was to characterize clinical and pathomorphological boundaries of MmD in our material.

Patients and methods

Patients

After revision of medical charts and all muscle biopsies analyzed in our laboratory from 1976 to 2004, we selected a group of 17 congenital myopathy patients with MmD. This group consisted of 8 males and 9 females. Our patients belonged to 14 families. The main inclusion criteria were clinical features of congenital myopathy and the presence of minicores on EM examination (Table I).

Methods

A formal cardiology assessment including echocardiography was performed in the majority of patients. Respiratory function was assessed clinically and by spirometry.

Open muscle biopsy had already been performed in all patients. Three patients had repeated muscle biopsy, and one patient had three muscle biopsies (the third one was taken during spine surgery). Muscle biopsies were taken from quadriceps (13), biceps (7), deltoid (1) and paraspinal muscle (1). In total, 22 muscle biopsies were analyzed.

Serial frozen sections for light microscopy were stained according to standard techniques [5].

For the purpose of this study all biopsies were reevaluated by electron microscopy. Muscle specimens were fixed in buffered glutaraldehyde and embedded in Spurr resin for thick and ultra-thin sections according to routine techniques.

Table I. Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Clinical features of congenital myopathy (early onset, proximal as well as distal weakness)</td>
<td>• Peripheral nerve involvement on electromyography</td>
</tr>
<tr>
<td>• Creatine kinase level &lt;2 times normal</td>
<td>• Morphological evidence of fibre necrosis and/or regeneration, glycogen or lipid storage, inflammatory infiltrates neurogenic changes as type grouping or atrophic angulated fibres</td>
</tr>
<tr>
<td>• Multiple minicores in more than 20% of muscle fibres by electron microscopy</td>
<td></td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>No.</th>
<th>Inheritance</th>
<th>Sex</th>
<th>Onset</th>
<th>Presentation</th>
<th>Actual functional status</th>
<th>Distribution of weakness</th>
<th>Dysmorphic features, others</th>
<th>Spinal involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s M</td>
<td>childhood</td>
<td>weakness of the lower limbs, early contractures</td>
<td>wheelchair</td>
<td>proximal</td>
<td>h-a p, spinal rigidity, talipes equinovarus</td>
<td>severe scoliosis, sr and limitation in cervical spine mobility, hyperlordosis</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>s M</td>
<td>childhood</td>
<td>early contractures</td>
<td>ambulant</td>
<td>proximal</td>
<td>thorax deformity</td>
<td>severe scoliosis, discrete sr and limitation in cervical spine mobility</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>s F</td>
<td>infancy</td>
<td>mdd, early contractures</td>
<td>ambulant</td>
<td>mainly distal</td>
<td>talipes equinovarus, early contractures</td>
<td>spina bifida within S1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>s F</td>
<td>infancy</td>
<td>mdd</td>
<td>ambulant</td>
<td>proximal, facial</td>
<td>h-a p, thorax deformity</td>
<td>mild scoliosis</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>s M</td>
<td>neonatal</td>
<td>h, mdd</td>
<td>ambulant</td>
<td>proximal, facial</td>
<td>h-a p</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>s M</td>
<td>neonatal</td>
<td>h, mdd</td>
<td>ambulant</td>
<td>proximal, facial</td>
<td>h-a p, prognathism</td>
<td>mild scoliosis</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>ar F</td>
<td>neonatal</td>
<td>h, mdd</td>
<td>ambulant</td>
<td>proximal, facial</td>
<td>h-a p, winged scapulas</td>
<td>moderate scoliosis, sr and limitation in cervical spine mobility</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>ar F</td>
<td>infancy</td>
<td>mdd</td>
<td>ambulant</td>
<td>proximal</td>
<td>none</td>
<td>mild scoliosis</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>ad M</td>
<td>childhood</td>
<td>weakness of the lower limbs</td>
<td>ambulant</td>
<td>mainly distal, facial</td>
<td>h-a p, talipes planus</td>
<td>mild scoliosis</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>ad F</td>
<td>childhood</td>
<td>mdd</td>
<td>ambulant</td>
<td>mainly distal</td>
<td>h-a p, talipes arcuratus</td>
<td>mild scoliosis</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>ad F</td>
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<td>weakness of the lower limbs</td>
<td>ambulant</td>
<td>mainly distal</td>
<td>h-a p</td>
<td>mild scoliosis</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>s F</td>
<td>neonatal</td>
<td>h, mdd</td>
<td>ambulant</td>
<td>proximal</td>
<td>none</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>s M</td>
<td>infancy</td>
<td>mdd, early contractures</td>
<td>ambulant</td>
<td>proximal</td>
<td>h-a p, early contractures</td>
<td>hyperlordosis</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>s M</td>
<td>infancy</td>
<td>mdd</td>
<td>ambulant</td>
<td>proximal</td>
<td>thorax deformity, h-a p, winged scapulas</td>
<td>severe scoliosis, sr and limitation in cervical spine mobility</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>s F</td>
<td>infancy</td>
<td>mdd</td>
<td>ambulant</td>
<td>proximal</td>
<td>h-a p</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>s M</td>
<td>neonatal</td>
<td>h, mdd</td>
<td>ambulant</td>
<td>proximal</td>
<td>hypogenitalism, overweight, mentally retarded</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>s F</td>
<td>neonatal</td>
<td>respiratory insufficiency, h, mdd</td>
<td>ambulant</td>
<td>proximal</td>
<td>h-a p, thorax deformity</td>
<td>mild scoliosis</td>
<td></td>
</tr>
</tbody>
</table>
Results

The main results are summarized in Tables II and III.

Inheritance and performed genetic investigations

Seventeen cases were identified in 14 families. Twelve cases were sporadic (patients 1-6, 12-17). In a family of 2 siblings (patients 7 and 8) the mode of inheritance was probably autosomal-recessive (AR). Three other patients (9-11) belonged to the same family. In this family two more members were affected (brother and father of the patient 9), but they were not included in the study because they did not have a muscle biopsy. Preliminary genetic investigations in this family revealed a potential linkage to the RYR1 gene. An autosomal-dominant (AD) trait is suspected.

<table>
<thead>
<tr>
<th>No.</th>
<th>Muscle biopsy at age [years]</th>
<th>Biopsied muscle</th>
<th>Light microscopy</th>
<th>Electron microscopy (multi-minicores)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>quadriceps</td>
<td>CFTD⁺</td>
<td>multi-minicores ++</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>deltoid</td>
<td>CFTD⁺, scanty minicores, artefacts</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>quadriceps</td>
<td>CFTD⁺</td>
<td>multi-minicores +</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>quadriceps</td>
<td>type 2 atrophy</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>quadriceps</td>
<td>CFTD⁺, minicores</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>quadriceps</td>
<td>CFTD⁺</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>biceps</td>
<td>CFTD⁺ + centrally located nuclei in 40% of fibres, minicores</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>biceps</td>
<td>CFTD⁺ + centrally located nuclei in 30% of fibres, minicores</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>quadriceps</td>
<td>CFTD⁺, minicores</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>quadriceps</td>
<td>CFTD⁺, minicores</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>11</td>
<td>37</td>
<td>biceps</td>
<td>type 2 predominance, few minicores</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>quadriceps</td>
<td>minicores</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>quadriceps</td>
<td>CFTD⁺, minicores</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>14</td>
<td>12</td>
<td>biceps</td>
<td>minicores</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>quadriceps</td>
<td>minicores</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>16</td>
<td>12</td>
<td>quadriceps</td>
<td>minicores</td>
<td>multi-minicores +++</td>
</tr>
<tr>
<td>17</td>
<td>8</td>
<td>biceps</td>
<td>CFTD⁺, minicores</td>
<td>multi-minicores +++</td>
</tr>
</tbody>
</table>

* CFTD (congenital fibre type disproportion, Brooke 1973) – type 1 hypoplasia and predominance, †multi-minicores + – 20-30%, ++ – 30-40%, +++ – >50%
Clinical features

Presentation

The first manifestation of disease was noted at birth in 6 cases, and during infancy or early childhood in the majority of cases. In this second group the predominant mode of presentation was developmental motor delay (7/17 patients). Congenital deformities such as talipes equinovarus (2 cases) or other dysmorphic features such as high-arched palate (12/17 cases) were also observed. In one patient, early contractures were the first noted manifestations of the disease.

Distribution of weakness

Most patients showed generalized muscle weakness and atrophy, which was clearly predominant in axial muscles. Trunk and particularly neck flexors were the most severely affected muscle groups. Distribution of weakness in limbs was predominantly proximal. In 4 cases there was marked distal wasting affecting mainly the lower limbs. Mild facial weakness was present in 6 cases. There was no limitation of eye movement in any case. Bilateral ptosis was observed in 2 cases.

Progression

Most cases were static or slowly progressive. One patient became a wheelchair user at the age of 10 because of progressing scoliosis and severe hip contractures (case 1). Two patients (cases 4 and 6) died in childhood of respiratory insufficiency during pneumonia. One patient died at the age of 42 (case 11) of unknown cause.

Contractures

Limb joint contractures were neither frequent nor severe. They involved mainly ankles, sometimes hips, knees and elbows. Marked ligamentous laxity was a prominent finding in 5 patients, often manifested as foot deformities and lumbar hyperlordosis. Most frequently, various spinal abnormalities were noted in our group of patients (11/17). Onset of scoliosis in infancy (before 6 years of age) was seen in two cases. Evident spinal rigidity and limitation in cervical spine mobility were present in 4 patients.

Other complications

Cardiac involvement was infrequent among our patients. ECG was normal in all patients. Echocardiography performed in 8 patients did not reveal any significant abnormalities. One patient had a mitral valve prolapse.

Spirometry was performed in 5 patients and showed restriction in 4 examined cases (cases 1, 9, 10 and 13). In the other patient spirometry did not reveal any abnormality at the age of 12 (case 16). Transient respiratory insufficiency during pneumonia was reported in patient 14 at the age of 10 years, and during the neonatal period in patient 17.

Five patients from our cohort underwent various surgical operations. No evidence of malignant hyperthermia during general anaesthesia was noted.

Clinical phenotypes

According to clinical classification, the „classical” phenotype of MmD comprising marked axial weakness and scoliosis with spinal rigidity was the most common in our group (11/17 patients). Respiratory function impairment was rare and occurred in 6 patients (cases 1, 4, 6, 9, 10 and 13). In two cases antenatal onset with arthrogryposis (talipes equinovarus) was noted. The moderate form of MmD, characterized by distal weakness and muscle wasting, was present in 4/17 cases (3 of them belonged to the same family). Scoliosis was generally mild in the majority of our patients but 3 cases of severe scoliosis were also noted.

Laboratory investigations

CK level was normal in all examined cases. EMG was performed in all patients. In 12 cases, EMG showed myopathic pattern such as low amplitude, polyphasic motor unit potentials and lack of spontaneous activity. Normal EMG pattern was found in 5 patients (2, 9, 10, 15 and 16). Motor nerve conduction velocities were normal in all cases.

Histopathological features

Multifocal lack of oxidative activity in the majority of both type 1 and 2 fibres (Fig. 1) was visible on light microscopy in 16/22 muscle biopsies and in 13/17 patients. In contrast, in all but one muscle biopsy, multi-minicores were seen on electron microscopy (Fig. 2 and Fig. 3). This means that in 4 cases first light microscopic analysis failed to reveal multi-minicores (patients 1, 3, 4, 6). Patient 1, who had 3 muscle biopsies at 10-year intervals, had never had multi-minicores visible on light
microscopy. Interestingly, they were found on electron microscopy only in the first and third muscle biopsies (from left quadriceps and paraspinal muscle, respectively). In 13 cases, the first muscle biopsy showed evident multi-minicores on light microscopy.

Fibre type disproportion was seen in the majority of biopsies with the association of type 1 predominance and hypertrophy as the most common finding (14/22 muscle biopsies). In addition, other patterns such as type 2 fibre atrophy or predominance were seen in 3 muscle biopsies.

Abundant (in 30-40% of fibres) centrally located nuclei were noted in 3 muscle biopsies from 2 siblings (7, 8). After preliminary assessment on light microscopy, centronuclear myopathy was suspected in both cases. EM examination revealed multiple small zones of sarcomeric disorganization.

There was no clear correlation between the entity of the morphological abnormalities and the severity of clinical involvement.

Discussion

We reviewed 17 clinically and morphologically defined cases of multi-minicore myopathy. The majority of cases were sporadic, but in two families AR and AD mode of inheritance can be postulated. Sporadic or AR forms seem to be those most frequently described in the literature. In contrast, autosomal dominant inheritance is very rare and has previously been reported only in a few families [24,34,36].

Onset of clinical symptoms in our series appeared to be comparable with those previously reported.

We found marked axial muscle involvement as the most prominent feature in the majority of cases but pronounced distal muscles weakness and mild facial involvement was also present in our group. These features have already been described in MmD [10,19,24,26,32,34,36].

External eye movements were normal in our series, in contrast with previous reports by other authors [11,32,30].

Fig. 1. Open biopsy from patient 16 stained for NADH-diaphorase, showing local loss of activity in minicores in several fibres (magnification 200 x)
Progressive joint contractures other than tightness of the Achilles tendon were rare. According to the phenotype analysis of 38 cases presented by Ferreiro et al. [7], scoliosis seems to be more frequent and severe in MmD than in any other congenital myopathies, and it can show steady evolution even when limb muscles weakness remains stable. In our group scoliosis was detected in 10 patients with progression requiring surgical intervention in two of them. Spinal rigidity was observed in 4/17 cases and it was rather mild. However, severe cases of spinal rigidity have been reported in the literature [2,34].

Mitral valve prolapse was the only cardiac structural defect observed in one of our patients, and it has also been reported by others in MmD [23].

Respiratory insufficiency can be frequent and severe in patients with MmD. Respiratory failure is usually multifactorial and includes respiratory muscle weakness, diaphragm involvement, thoracic wall deformities and scoliosis. In our group 2 patients died of respiratory insufficiency and in 2 other cases transient respiratory function impairment was reported. Additionally, mild restriction of respiratory function on spirometry was present in 4 examined cases.

In considering muscle pathology, analysis of biopsies in our group revealed that light microscopy is not the most reliable method for the diagnosis of MmD. It has already been reported that in some cases minicores may not be visible on light microscopy [7,15]. It is suggested [4] that in some affected individuals with MmD, as well as nemaline myopathy and centronuclear myopathy, the hallmark pathological features are not found in the biopsy due

![Fig. 2. Transversely sectioned fibres from patient 16 on electron microscopy. Electron-dense material of Z-line origin forming irregular zones (magnification 20 000 x)](image-url)
to sampling error or due to the young age of the patient. In MmD in early childhood, the pathological changes in muscle can be infrequent and small, sometimes represented only by some Z-line streaming. It is of special interest that in our group 4 patients were initially diagnosed with minimal change myopathy. All of them were less than 6 years old and this fact can possibly explain the lack of evident characteristic changes on light microscopy. The absence of multi-minicores in the second muscle biopsy in patient 1 is difficult to explain; perhaps sampling error occurred in that case. Three of our patients (1, 3, 6) presented morphological changes diagnosed as congenital fibre type disproportion (CFTD) – a structural myopathy described by Brooke in 1973 [19]. It is now clear that care must be taken to exclude other conditions that can share CFTD’s histological pattern on light microscopy.

In addition, the coexistence of MmD lesions with cores [3,29,33] and rods [27] or centrally located nuclei [10,12,20] points to the difficulties of defining morphological boundaries between different types of congenital myopathies. This kind of problem arose when muscle biopsies of two siblings (patients 7 and 8) were analyzed. These patients were initially diagnosed with centronuclear myopathy. In these cases, EM examination was essential to establish appropriate diagnosis of MmD.

In conclusion, MmD is a morphologically defined muscle disorder in which characteristic histological changes may not be clearly visible on LM. According to our observations and in the absence of genetic diagnosis, it seems that EM examination is essential in the diagnostic process of MmD and for this reason it should be performed in all patients with myopathy.

Fig. 3. Longitudinally sectioned fibres from patient 16 on electron microscopy. Severe focal disorganization of the myofibrillar structure and mitochondrial depletion (magnification 20 000 x)
and fibre type disproportion or centrally located nuclei as the only pathology on light microscopy, possibly targeting an appropriate genetic investigation.

Disclosure: the authors report no conflicts of interest.

References


