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Histopathological differences of myotonic dystrophy type 1 (DM1) and PROMM/DM2

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Abstract—Muscle biopsy findings in DM2 have been reported to be similar to those in DM1. The authors used myosin heavy chain immunohistochemistry and enzyme histochemistry for fiber type differentiation on muscle biopsies. Their results show that DM2 patients display a subpopulation of type 2 nuclear clump and other very small fibers and, hence, preferential type 2 fiber atrophy in contrast to type 1 fiber atrophy in DM1 patients.

Muscle biopsies. Deltoit muscle biopsies were obtained from three French DM2 patients, two of whom originated from France and one from Serbia. Detailed clinical features of one patient (F3) from the series have been previously reported. Vastus lateralis muscle biopsies from three patients from two unrelated Finnish DM2 families not previously reported were examined (FIN1 and FIN3 are cousins). Biceps brachii muscle biopsies were obtained from three patients from unrelated Italian DM2 families. Control subjects were age-matched and mutation-verified DM1 patients from different families (deltoid, three; vastus, three; biceps, two), with similar muscle weakness shown on muscle biopsy, and one recessive congenital myotonia patient with homozygous R94X mutation in the CLCN1 gene.

Histologic procedure. Serial transverse muscle cryostat sections 6 μm and 10 μm thick were cut for immunohistochemical (IHC) and routine histochemical stainings, respectively, with hematoxylin and eosin (H&E), Gomori’s trichrome, and nicotinamide adenine dinucleotide (NADH) tetrazolium reductase. A standard myofibrillar ATPase staining protocol was used after preincubation at pH 4.3, pH 4.6, and pH 9.4 or 10.4. Ventana Nexes automated immunostainer (Ventana Medical Systems, Tuscon, AZ) was used for IHC, with avidin-biotin-complex method followed by diaminobenzidine (DAB) detection. Monoclonal primary antibodies against different myosin heavy chain (MHC) isotypes were used at the following dilutions: MHCfast, 1:400 (My-32, Sigma Chemical Co., St. Louis, MO); MHCslow, 1:5,000 (NOQ7.5.4D, Sigma); and MHCd (developmental), 1:10 (RMN2/9D2, Novocastra Laboratories, Newcastle upon Tyne, UK). Quantitative evaluation of fiber diameter was made with Scion Image (Scion Corporation, Frederick, MD) on images taken with a microscope (3.2 × 10 original magnification) and sections stained with slow and fast MHC IHC. The size of muscle fibers was assessed by measuring the "smallest fiber diameter." The number of muscle fibers evaluated in each sample ranged from 537 to 5,151 (see supplementary content on the Neurology Web site). All data were analyzed in triplicate.

Patients and methods. Deltoit, biceps brachii, and vastus lateralis muscle biopsies from nine patients from three European countries were re-examined. All had PROMM phenotype and expanded DM2 alleles in the range of 5 to 25 kb as assessed by Southern analysis. The biopsies were previously taken for diagnostic purposes and used for this study after informed consent from the patients. The Table provides a description of clinical features of the DM2 patients in this study. Electromyography (EMG) showed myotonia for all patients.
elaborated using Microcal Origin (Microcal Software Inc., Northampton, MA). The metahistograms were normalized to normal mean diameter for men and women. Atrophy and hypertrophy factors were also calculated.¹⁰

**Results.** Common histopathologic findings. Routine histochemical stainings of DM2 muscle sections showed myopathic changes, including increased fiber size variation and internalized nuclei (figure 1). Increases of connective tissue, fat replacement, or necrotic fibers were rarely observed. Structural changes mainly consisted of occasional moth-eaten fibers, whereas subsarcomeral masses and ring fibers were particularly rare. Occasional rimmed vacuolar fibers or ragged-red fibers (RRF) were encountered in some specimens. Central nuclei were frequent in DM1 and DM2 biopsies, and many small fibers with nuclear clumps were observed in most DM2 specimens (see figure 1). Rounded or thin angular atrophic fibers were usually scattered, whereas fiber type grouping or targetoid fibers were not encountered.

![Figure 1](image1.png)

**Figure 1.** Panel showing vastus lateralis histopathology of DM2 compared with DM1. (A through D) Finnish DM2 Patient FIN1. (E and F) Italian DM1 patient. (A) Hematoxylin and eosin (H&E); (B) ATPase pH 10.4; (C) Immunohistochemical (IHC), MHCslow; (D) IHC, MHCfast; (E) IHC, MHCslow; and (F) IHC, MHCfast. The population of small type 2 fibers in DM2 muscle is more distinguishable when IHC staining is used instead of ATPase. In the DM1 control muscle, no population of small fibers of either type is present. Arrows indicate nuclear clump fibers. Scale bar = 100 μm.

**Table** Clinical findings of the DM2 patients included in this study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>M/F</th>
<th>Age</th>
<th>Onset age</th>
<th>First symptom</th>
<th>Clinical muscle signs</th>
<th>Systemic signs</th>
<th>Other findings</th>
<th>Biopsied muscle (MRC score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>French</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>M</td>
<td>49</td>
<td>40</td>
<td>Wk</td>
<td>Wk</td>
<td>Ca, B, Hy</td>
<td>—</td>
<td>—</td>
<td>Deltoid (4)</td>
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<tr>
<td>F3</td>
<td>M</td>
<td>45</td>
<td>41</td>
<td>Wk</td>
<td>Wk, P</td>
<td>Ca, B, D, IR, Hy, G</td>
<td>At, HCh</td>
<td>—</td>
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<tr>
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<td>M</td>
<td>57</td>
<td>55</td>
<td>P</td>
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<td>Ca, Hy</td>
<td>Tr</td>
<td>—</td>
<td>Deltoid (5)</td>
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<td></td>
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<tr>
<td>FIN1</td>
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<td>39</td>
<td>36</td>
<td>CK</td>
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<td>Ca</td>
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<td>34</td>
<td>27</td>
<td>St</td>
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<td>—</td>
<td>—</td>
<td>Vastus lateralis (5)</td>
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<tr>
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<td>Ca</td>
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<td>Vastus lateralis (5)</td>
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<tr>
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<td>F</td>
<td>60</td>
<td>40</td>
<td>Wk</td>
<td>Wk, My</td>
<td>Ca</td>
<td>—</td>
<td>Biceps brachii (4)</td>
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</tr>
<tr>
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<td>M</td>
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<td>50</td>
<td>Wk</td>
<td>Wk, My</td>
<td>Ca</td>
<td>—</td>
<td>Biceps brachii (5)</td>
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<tr>
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<td>59</td>
<td>45</td>
<td>Wk, P</td>
<td>Wk</td>
<td>Ca</td>
<td>—</td>
<td>Biceps brachii (5)</td>
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</tbody>
</table>

At = severe atherosclerosis; B = balding; Ca = cataracts; CK = serum CK elevation; D = diabetes mellitus; G = elevated gamma glutamyltransferase; HCh = hypercholesterolemia; Hy = hyperhidrosis; IR = insulin resistance; My = clinical myotonia; P = pain; Pt = ptosis; St = stiffness; Tr = tremor; Wk = weakness.
Fiber type analysis. The metahistograms based on IHC data in figure 2 show preferential fiber type 2 atrophy in vastus lateralis, biceps, and deltoid muscles from DM2 patients. Hypertrophy of type 2 fibers in biceps of DM2 patients is also evident. In DM1 patients, marked atrophy of type 1 fibers and slight atrophy of type 2 fibers are present in all muscles. A control sample from the recessive congenital myotonia patient revealed no preferential type 2 atrophy (data not shown). A subpopulation of small fibers (diameter ≤ 20 μm), including all the nuclear clump fibers (which expressed almost exclusively fast MHC [type 2 fiber] isoform), was seen in the DM2 biopsies. Nuclear clumps and other small type 2 fibers were prominent in some vastus lateralis and deltoid samples even at onset of symptoms. IHC and the ATPase method gave identical fiber type distribution patterns for fibers more than 40 μm in diameter, but many fibers less than 20 μm in diameter, well-identified by MHC IHC, were not readily discernible with H-E and ATPase (see figure 1). A small proportion of fibers of all sizes expressed fast and slow MHC isoforms, and few regenerating fibers expressing the developmental myosin isotype were observed. The presence of 2B fibers was confirmed in all DM2 biopsies in this study, although not in constant proportions (see table E-1 on the Neurology Web site for a summary of the key figures of the histographic analysis). The calculated atrophy and hypertrophy factors are in full agreement with the results derived from other methods in this study.

Discussion. Our study shows preferential type 2 fiber atrophy in DM2 and absence of type 1 fiber atrophy in contrast to DM1. IHC determination of fiber type distribution revealed many small fibers (diameter ≤ 20 μm), including the nuclear clump fibers, practically all of which were identified as type 2 fibers. This study showed a variable fiber type distribution in DM2 depending on muscle site. In particular, the previously described type 1 fiber predominance was only attested in deltoid biopsies and was not confirmed in vastus lateralis and biceps brachii muscles. Hypertrophy was variable in both fiber types, which may be the result of secondary compensation or myotonia.

Further studies will be required to determine the fiber type of nuclear clump fibers appearing in more
severely affected DM1 muscles and whether the different extremely small type 2 fibers in DM2 belong to different subpopulations.

Muscle biopsy is rarely needed for DM1 diagnosis, but for DM2 the situation is different because the clinical findings are milder and less pathognomonic. The histopathologic findings reported here might help the diagnostic procedure to select patients for further DM2 molecular genetic verification.

Acknowledgment
The authors thank Dr. M. Fardeau, who initiated the morphologic study in the French group, and Dr. J. Pouget, who is actively involved in the French PROMM study.

References

A missense mutation in the mitochondrial ND5 gene associated with a Leigh-MELAS overlap syndrome

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Abstract—A 13084 A->T missense mutation in the mitochondrial ND5 gene was identified in a 16-year-old boy affected with a progressive neurodegenerative disorder combining features of Leigh and MELAS (mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes) syndromes. Muscle biopsy analysis revealed partial complex I deficiency. The mutation presented a variable degree of heteroplasmy in the patient’s tissues. This finding underlines the contribution of mtDNA-encoded complex I subunits in the etiology of complex I deficiency associated with encephalopathy.

Isolated complex I deficiency is a common enzyme defect among the group of oxidative phosphorylation disorders.1 Human complex I consists of at least 36 nuclear DNA (nDNA)- and 7 mitochondrial DNA (mtDNA)-encoded subunits. Leigh syndrome (LS) or Leigh-like disease is the most common phenotype associated with an isolated complex I deficiency, representing up to 50% of total cases, although isolated myopathies, MELAS (mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes) syndromes, and Leber’s hereditary optic neuropathy (LHON) presentations are also described.1

Here we report a novel ND5 heteroplasmic missense mutation associated with severe progressive infantile encephalopathy, with features of LS and MELAS. The proband’s mother, carrying a lower mutation level, presents recurrent migraine attacks and bilateral optic atrophy. These data expand the molecular etiology of partial isolated complex I deficiency.

† Deceased.

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