ABSTRACT

Objective: There are currently no effective treatments to halt the muscle breakdown in Duchenne muscular dystrophy (DMD), although genetic-based clinical trials are being piloted. Most of these trials have as an endpoint the restoration of dystrophin in muscle fibers, hence requiring sufficiently well-preserved muscle of recruited patients. The choice of the muscles to be studied and the role of noninvasive methods to assess muscle preservation therefore require further evaluation.

Methods: We studied the degree of muscle involvement in the lower leg muscles of 34 patients with DMD >8 years, using muscle MRI. In a subgroup of 15 patients we correlated the muscle MRI findings with the histology of open extensor digitorum brevis (EDB) muscle biopsies. Muscle MRI involvement was assigned using a scale 0–4 (normal–severe).

Results: In all patients we documented a gradient of involvement of the lower leg muscles: the posterior compartment (gastrocnemius > soleus) was most severely affected; the anterior compartment (tibialis anterior/posterior, popliteus, extensor digitorum longus) least affected. Muscle MRI showed EDB involvement that correlated with the patient’s age (p = 0.055). We show a correlation between the MRI and EDB histopathologic changes, with MRI 3–4 grades associated with a more severe fibro-adipose tissue replacement. The EDB was sufficiently preserved for bulk and signal intensity in 18/22 wheelchair users aged 10–16.6 years.

Conclusion: This study provides a detailed correlation between muscle histology and MRI changes in DMD and demonstrates the value of this imaging technique as a reliable tool for the selection of muscles in patients recruited into clinical trials. Neurology® 2011;76:346-353

GLOSSARY

AO = antisense oligonucleotide; DMD = Duchenne muscular dystrophy; EDB = extensor digitorum brevis; KAFO = knee-ankle-foot orthosis; NSA = number of signal averages; TE = echo time; TR = repetition time.

The progressive loss of muscle fibers and their replacement by fat and connective tissue is part of the disease course of DMD. Despite several exhaustive natural history studies on the clinical course of DMD, the progression of pathology in individual muscle groups is poorly defined. Information on muscle pathology in DMD is limited to the muscles that are biopsied at the time of diagnosis (often quadriiceps femoris), the mean age at diagnosis being ~4.5 years.

There are now early clinical trials using novel genetic-based techniques in boys with DMD with the aim to restore dystrophin expression. These include the drug PTC124, which allows...
read-through of nonsense mutations, and antisense oligonucleotides (AOs), which induce exon skipping and restore the reading frame in boys with out-of-frame deletions. These early trials require selection of sufficiently well-preserved muscles for the evaluation of induced dystrophin production, which is the primary outcome assessed. Recently MRI has been used for quantification of atrophy of the small foot muscles in chronic patients with diabetic neuropathy.

We have recently completed a phase Ib/IIa clinical trial showing restoration of dystrophin expression by using IM injection of a morpholino AO (AVI-4658) in a group of boys with DMD with deletions that can be rescued by skipping exon 51.

In order to make an informed selection of the muscle to inject in this trial, we assessed the degree of muscle involvement of leg and foot muscles in patients with DMD using muscle MRI. In a subset of patients we correlated muscle MRI findings with histopathologic analysis of open muscle biopsies from the extensor digitorum brevis muscle (EDB), taken under anesthesia at the time of planned orthopedic surgery. We hypothesized that MRI findings would correlate with muscle histopathology, and this correlation in DMD is not well-established. We also correlated MRI changes of the EDB muscles with the age and time in years since loss of independent ambulation for those patients who were wheelchair-dependent.

**METHODS**

The two sites (Dubowitz Neuromuscular Centre in London and Newcastle University) involved in this preparatory work are two of the major centers for care of pediatric neuromuscular patients in the United Kingdom. Both sides are part of the UK MDEX consortium (http://www.mdex.org.uk/), involved in experimental therapy for DMD. This preparatory study was approved by a Multicenter Ethics Committee in the United Kingdom.

All boys with DMD studied with muscle MRI were over 8 years of age and had their diagnosis established by either DNA mutation analysis or muscle biopsy, or both. In a subset of boys undergoing elective surgery, we obtained a muscle biopsy of the EDB muscle. We excluded patients with severe cardiorespiratory involvement (forced vital capacity <25% predicted [according to the arm span or height] and a fractional shortening <25% or ejection fraction <40%) as this would have been a contraindication to planned surgery. We also excluded patients with severe cognitive dysfunction and inability to collaborate with the assessing procedure. Informed consent was obtained from patients older than 16 years. Patients younger than 16 years gave their assent, and consent was obtained from their parents/carers. We collected the following clinical information:

- Mobility status at the time of the study if still ambulant and age/years since loss of independent ambulation if not ambulant.
- Dystrophin gene mutation analysis.
- Reports from diagnostic muscle biopsies, detailing dystrophin expression.

**Muscle MRI.** As part of our study protocol, we performed a muscle MRI of the limb muscles (foot and below the knee) on all our patients. The boys did not receive any sedation and the total examination time was less than 30 minutes.

MRI sequences were optimized using volunteers to determine the best one for displaying the EDB muscle, using a conventional T1-weighted spin echo on either a 1.0-T or a 3-T Philips scanner system. Noncontrast enhanced images were obtained from both legs (below the knees and feet).

To study the distal leg muscles (below knees) we used a body coil for obtaining T1 spin echo, in the transverse plane, 15 slices each leg, thickness 5 mm each, and the gap between slices varied from 10 to 50 mm dependent on the site and on the size of the patient. The axial plane was selected with respect to the long axis of the body. A spin echo pulse sequence was used (repetition time [TR] = 500 msec; echo time [TE] = 20 msec) with a 256 × 256 matrix, number of signal averages (NSA) = 1, and a variable field of view of 25–50 cm.

To study the feet, we used a head coil with a closed end and a T1 volume-weighted sequence in the sagittal plane, to obtain approximately 120 slices, thickness 0.8 mm each, with a total acquisition time of 8 minutes. A spin echo pulse sequence was used (TR = 30 msec; TE = 4.5 msec) with a 256 × 156 matrix, NSA = 1, and a flip angle 30. Only one foot was placed in the coil each time.

For the muscle MRI below the knees, sections were generally analyzed within the midupper section of the lower legs, as musclebulk is greatest at this level and muscle abnormality could be more clearly visualized as a result. The EDB is a broad, thin intrinsic foot muscle located on the dorsum of the foot immediately under the skin. The EDB arises from the lateral talocalcaneal ligament and from the common limb of the cruciate crural ligament (figure 1). It attaches on the phalanges of the second to fifth toes. Care was taken to review in all muscles the remainder of the muscle bulk in order to confirm that the analyzed section was representative of the whole muscle.

Muscle MRI scans were assessed for normal and abnormal (atrophy/hypertrophy) muscle bulk and for normal and abnormal signal intensity within the different muscle groups and in particular the EDB muscle of both feet. All muscle MRI scans were assessed by 2 independent observers (M.K., M.R.), and scored as follows:

- **Stage 0:** Normal appearance
- **Stage 1:** Scattered small areas of increased density
- **Stage 2a:** Numerous discrete areas of increased density less than 30% of the volume of the muscle
- **Stage 2b:** Numerous discrete areas of increased density with early confluence, 30%–60% of the volume of the muscle
Stage 3: Washed-out appearance due to confluent areas increased density with muscle still present at the periphery

Stage 4: End-stage appearance, muscle entirely replaced by areas of increased density

EDB open muscle biopsy. An open biopsy of the EDB muscle was obtained within 0–8 months form the muscle MRI in 16 patients who underwent planned surgery. The biopsies were obtained either at the time of surgery to release contractures of the Achilles tendons or improve foot deformities (triple foot arthrodesis) when older than 10 years of age or during spinal surgery. The latter is usually performed at around 13–14 years of age or later. The remaining patients did not undergo EDB biopsy. For the EDB biopsy, an incision was drawn longitudinally between the lateral malleolus and the tuberositas of the fifth metatarsal bone following clinical examination. EDB muscle biopsy specimens were investigated in our routine diagnostic pathology laboratory. Muscle histology was scored by 2 independent observers (C.A.S., L.F.) in transverse sections; the observers scored separately and reached agreement after discussion, according to the following protocol\textsuperscript{14}:

Grade 1: Retention of fascicular pattern with no obvious proliferation of fat or connective tissue

Grade 2: Retention of fascicular pattern plus invasion by connective tissue and/or fat proliferation

Grade 3: Disruption of muscle fascicles with marked connective tissue and/or fat proliferation

Grade 4: Severe change with replacement of more than 50% of muscle by fat and connective tissue

Considering that the observed MRI changes could have also been influenced by fatty infiltration of the EDB muscle, we scored the EDB fat content according to the following criteria:

Grade 1: Only single adipocytes visible, well-preserved fascicles

Grade 2: Perimysial adipocytes visible with preserved fascicle structure

Grade 3: Numerous perimysial and endomysial adipocytes with loss or partial loss of fascicular structure

Grade 4: Pronounced fatty replacement throughout with fascicle structure lost

Genetic mutations. The endpoints of the deletions in the DMD gene were defined in all patients using genomic DNA isolated from blood. Most patients had out-of-frame deletions, 3 had nonsense mutations, and one a splice site mutation leading to an out-of-frame RNA deletion.

Statistics. Descriptive statistics were carried out wherever appropriate by using the Graph Pad prism 4 program. We also correlated the changes in the MRI of the EDB muscles with the age of the patient and the years since loss of independent ambulation. Both correlations were tested using the Spearman rank order correlation coefficient.

We also correlated the scoring of EDB muscles using both MRI and histopathologic grades using the Spearman rank coefficient $r_s$.

RESULTS Thirty-four patients with DMD participated in this study. Thirty-two of them had muscle MRI investigations of both lower legs and both feet; one patient did not have an MRI of his feet and a further patient did not have an MRI of his calves. Fifteen patients (mean age 12.16 ± 2.15 years) also had EDB biopsies within 0–8 months (2 ± 2) from their muscle MRI studies, allowing the correlative MRI and EDB studies. One patient had an EDB biopsy (subject 1) but did not have a foot MRI so correlation was not possible. The table shows the overall population studied in relation to their age, mobility, DMD gene mutation, and EDB biopsies obtained with scoring of EDB muscles using both MRI and histopathologic scores.

MRI. Both observers (M.K., M.R.) had a training session ahead of scoring the muscle MRI scans. Interobserver agreement was reached in all subjects.

Calf muscles. The posterior compartment was most severely affected (gastrocnemius > soleus). The anterior compartment was least affected (tibialis anterior/posterior, popliteus, extensor digitorum longus) (figure 2). The state of preservation of the anterior compartment muscles and in particular the tibialis anterior/posterior was similar to the EDB in all patients studied ($n = 33$). Conversely, the state of preservation of the posterior muscles (gastrocnemius > soleus) was always more advanced than the EDB muscles in all patients studied ($n = 33$).

Foot muscles. The mean age of the 33 patients studied was 12.51 ± 2.47 years (table). Muscle MRI showed that the EDB muscles were always identifiable despite their small dimensions and had variable levels of signal changes (figure 2). Five patients were either using knee-ankle-foot orthoses (KAFOs, $n =$
<table>
<thead>
<tr>
<th>Subjects studied</th>
<th>Age at study</th>
<th>Functional abilities</th>
<th>MRI muscle</th>
<th>MRI EDB changes</th>
<th>Dystrophin gene mutation</th>
<th>EDB muscle biopsy scoring histology score/fat score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13 y</td>
<td>WC since 9 y</td>
<td>BK</td>
<td>NA</td>
<td>Stop mutation exon 70 (c.10171C&gt;T; p.R3391X)</td>
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<tr>
<td>2</td>
<td>11 y 3 mo</td>
<td>WC since 11 y 2 mo</td>
<td>EDB and BK</td>
<td>Grade 2b</td>
<td>Del 45-52</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>9 y 10 mo</td>
<td>KAFOs standing since 8 y</td>
<td>EDB and BK</td>
<td>Grade 2b</td>
<td>Del 46-50</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>9 y</td>
<td>Walking with difficulties</td>
<td>EDB and BK</td>
<td>Grade 1</td>
<td>Del 61</td>
<td>NA</td>
</tr>
<tr>
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<td>11 y 10 mo</td>
<td>WC since 11 y 5 mo</td>
<td>EDB and BK</td>
<td>Grade 3</td>
<td>Del 46-49</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>15 y</td>
<td>KAFOs standing since 10 y</td>
<td>EDB and BK</td>
<td>Grade 4</td>
<td>Del 46-50</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>9 y 8 mo</td>
<td>Standing since 9 y</td>
<td>EDB and BK</td>
<td>Grade 2a</td>
<td>Del 47-50</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>12 y 11 mo</td>
<td>WC since 10 y</td>
<td>EDB and BK</td>
<td>Grade 2b</td>
<td>Del 46-52</td>
<td>2/2</td>
</tr>
<tr>
<td>9</td>
<td>11 y 4 mo</td>
<td>WC since 7 y</td>
<td>EDB and BK</td>
<td>Grade 2b</td>
<td>Del 3-13</td>
<td>2/2</td>
</tr>
<tr>
<td>10</td>
<td>15 y 9 mo</td>
<td>WC since 12 y</td>
<td>EDB and BK</td>
<td>Grade 3</td>
<td>Stop mutation exon 53 (c.7720C&gt;T; p.Q2574X)</td>
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</tr>
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<td>Del 44</td>
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</tr>
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<td>Grade 2a</td>
<td>Del 51-53</td>
<td>2/2</td>
</tr>
<tr>
<td>13</td>
<td>11 y</td>
<td>WC since 9 y</td>
<td>EDB and BK</td>
<td>Grade 2b</td>
<td>Del 45-52</td>
<td>3/3</td>
</tr>
<tr>
<td>14</td>
<td>17 y 7 mo</td>
<td>WC since 11 y 8 mo</td>
<td>EDB and BK</td>
<td>Grade 4</td>
<td>Del 50</td>
<td>NA</td>
</tr>
<tr>
<td>15</td>
<td>12 y 3 mo</td>
<td>WC since 9 y 9 mo</td>
<td>EDB and BK</td>
<td>Grade 3</td>
<td>Del 46-51</td>
<td>3/3</td>
</tr>
<tr>
<td>16</td>
<td>12 y 5 mo</td>
<td>KAFOs walking 20 steps</td>
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<td>Del 45-52</td>
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</tr>
<tr>
<td>17</td>
<td>12 y 5 mo</td>
<td>Cruises indoors 5-10 steps</td>
<td>EDB and BK</td>
<td>Grade 2a</td>
<td>Del 46-50</td>
<td>NA</td>
</tr>
<tr>
<td>18</td>
<td>16 y</td>
<td>WC since 10 y</td>
<td>EDB and BK</td>
<td>Grade 2a</td>
<td>Stop mutation exon 50 (c.7437G&gt;A; p.E2460X)</td>
<td>NA</td>
</tr>
<tr>
<td>19</td>
<td>13 y 8 mo</td>
<td>WC since 11 y 10 mo</td>
<td>EDB and BK</td>
<td>Grade 3</td>
<td>Del 45</td>
<td>NA</td>
</tr>
<tr>
<td>20</td>
<td>12 y</td>
<td>WC since 11 y</td>
<td>EDB and BK</td>
<td>Grade 2b</td>
<td>Del 46-49</td>
<td>2/2</td>
</tr>
<tr>
<td>21</td>
<td>16 y 3 mo</td>
<td>WC since 11 y</td>
<td>EDB and BK</td>
<td>Grade 2a</td>
<td>c.7201-1_7201-14del Intron 49/splice site exon 50</td>
<td>2/3</td>
</tr>
<tr>
<td>22</td>
<td>12 y 9 mo</td>
<td>WC since 10 y 1 mo, but rides static bike 10 min daily</td>
<td>EDB and BK</td>
<td>Grade 2b=wf/ grade 3</td>
<td>Del 50</td>
<td>NA</td>
</tr>
<tr>
<td>23</td>
<td>16 y 7 mo</td>
<td>WC since 15 y 7 mo and supported standing 30 min/day</td>
<td>EDB and BK</td>
<td>Grade 2b</td>
<td>Del 45-50</td>
<td>NA</td>
</tr>
<tr>
<td>24</td>
<td>15 y 6 mo</td>
<td>WC since 8 y 2 mo</td>
<td>EDB and BK</td>
<td>Grade 4</td>
<td>Del 46-50</td>
<td>NA</td>
</tr>
<tr>
<td>25</td>
<td>15 y 4 mo</td>
<td>WC since 12 y</td>
<td>EDB and BK</td>
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<td>Del 48-50</td>
<td>NA</td>
</tr>
<tr>
<td>26</td>
<td>12 y</td>
<td>WC since 8 y 6 mo</td>
<td>EDB and BK</td>
<td>Grade 3=wf/ grade 2a</td>
<td>Del 45-50</td>
<td>NA</td>
</tr>
<tr>
<td>27</td>
<td>11 y 7 mo</td>
<td>WC since 10 y</td>
<td>EDB and BK</td>
<td>Grade 2b=wf/ grade 3</td>
<td>Del 48-50</td>
<td>3/3</td>
</tr>
<tr>
<td>28</td>
<td>15 y</td>
<td>Walks indoors</td>
<td>EDB and BK</td>
<td>Grade 2b=wf/ grade 2b</td>
<td>Del 48-50</td>
<td>2/2</td>
</tr>
<tr>
<td>29</td>
<td>10 y 9 mo</td>
<td>Walks independently</td>
<td>EDB and BK</td>
<td>Grade 1</td>
<td>Del 45-50</td>
<td>2/2</td>
</tr>
<tr>
<td>30</td>
<td>11 y 9 mo</td>
<td>Walks independently</td>
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<tr>
<td>31</td>
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<td>Walks independently</td>
<td>EDB and BK</td>
<td>Grade 2a</td>
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<tr>
<td>32</td>
<td>8 y 5 mo</td>
<td>Walks independently</td>
<td>EDB and BK</td>
<td>Grade 2b</td>
<td>Del 45-50</td>
<td>NA</td>
</tr>
<tr>
<td>33</td>
<td>12 y</td>
<td>Walks independently</td>
<td>EDB and BK</td>
<td>Grade 2b</td>
<td>Del 49-50</td>
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</tr>
<tr>
<td>34</td>
<td>10 y</td>
<td>Walks independently</td>
<td>EDB and BK</td>
<td>Grade 1</td>
<td>Del 49-50</td>
<td>2/2</td>
</tr>
</tbody>
</table>

Abbreviations: = − asymmetric extensor digitorum brevis involvement on muscle MRI grading; BK = below knee; EDB = extensor digitorum brevis; KAFO = knee-ankle-foot orthosis; NA = sample not obtained; WC = wheelchair.
3) or cruising/standing indoors \((n = 2)\) at the time of the study. A further 9 patients were walking independently, 3 with difficulties. Only 4 patients had severe end-stage changes of the EDB (grade 4). Two of them were 15.5 years and a further 1 17.6 years although a younger 10-year-old patient had similar changes (figure 2, table). The majority of the remaining patients had relatively good preservation of their EDB muscles (grades 2a–2b), including 5 patients older than 15 years. Two patients had very mild changes (grade 1) while they were still ambulant (figure 2). Six patients had asymmetric involvement of the EDB as judged by the MRI changes. Of those, 2/6 were able to walk indoors, 1 patient walked with KAFOs, and 3/6 patients were wheelchair users.

Correlation between MRI and clinical findings (age of the patient and years since loss of ambulation). The muscle MRI showed a progressive involvement of the EDB, which was more obvious in older patients and in those who had lost ambulation for a longer time. We observed a correlation of MRI score with increasing age (figure 3A) \((p = 0.055, n = 33, r_s = 0.29)\) and this was also reflected in the correlation between the time in years since loss of independent ambulation and EDB MRI changes, although this did not reach significance \((p = 0.18, n = 22, r_s = 0.2)\).

**EDB histology.** Fifteen of 16 patients had relatively good preservation of their EDB muscles on histology (grade 2, \(n = 10\), grade 3, \(n = 4\)). One patient older than 15 years had severe changes (grade 4) (table). Furthermore, we scored the fatty replacement in the EDB muscle separately as described above: in only 2 samples there was a difference between the fat score and the overall histology score (table).

**Correlation between MRI and EDB histopathologic findings.** There was a good correlation between the MRI and the EDB histopathology in 10/15 of the patients studied with both MRI and EDB biopsies (figure 3B) \((p < 0.001, n = 15, r_s = 0.812)\). The correlation between MRI scores and EDB fat score was also significant (figure 3C) \((p < 0.05, n = 15, r_s = 0.62)\). Muscle MRI and histology correlate studies shows that MRI grading 2–3 was associated with sufficiently preserved EDB structure (grade 2 in 11 patients and grades 2/3 or 3 in 3 patients). MRI grades 3 and 4 were associated with a more fibroadipose tissue replacement (figure 2, table).

**DISCUSSION** Muscle MRI is being increasingly used as a diagnostic tool as various inherited neuromuscular disorders show a specific pattern of muscle involvement.\(^{15}\) In DMD, T1-weighted images show a progressive and selective pattern of involvement with fatty infiltration of the lower limb muscles when boys are older than 6–7 years,\(^ {16,17}\) but correlative MRI–histologic studies are lacking. Our study was designed to ascertain the involvement of the EDB muscles in a population of boys with DMD using muscle MRI, and to obtain correlations between muscle MRI and histologic changes in a subset of these boys with DMD. This preparatory study was
also designed to validate muscle MRI as a tool to allow the selection of patients with sufficiently preserved EDB muscle for IM injection of an AO.11

Our results indicate that the EDB was relatively well-preserved in most patients, even in the nonambulant ones. All but four of our patients had sufficiently preserved (grade 1–3 changes) EDB muscles by MRI, despite 22 having lost the ability to walk for a period of 2.9 (±2) years. End-stage appearance (grade 4), with the EDB muscle being completely replaced by increased density connective tissue and fat, was however documented in 4 patients (2 of them were 15.5 years and a further 2 17.6 years and 10 years), all nonambulant. In the population of boys with DMD studied, there was a significant correlation between the EDB MRI grading and the years since loss of ambulation. Our data complement those of a previous study demonstrating a good correlation between MRI grading of the lower limb muscles (thighs and calves) and the patients' clinical function as well as their disease duration.18 Nevertheless, individual patients showed unexpected relative preservation (grade 2 changes) on MRI grading despite advanced age (mean age 16 years, mean 4 years since loss of ambulation). In 6 patients there was an asymmetric MRI involvement with regard to muscle bulk and structural changes, with 1 of the 2 EDB muscles being slightly better preserved (mostly grades 2a/2b). Asymmetric individual muscle involvement has been described in the lower limb muscles of patients with DMD in a single previous study, a finding due to the high resolution of muscle MRI.18 We did not observe any obvious asymmetric involvement in the transverse sections of the below the knees muscle groups in our cohort, although this is a phenomenon that can be found in female manifesting carriers of DMD.19 We have not found any patient with absent EDB muscles, which is a normal but very rare variation in the general population.20

The overall changes in the lower leg muscle groups were always more advanced compared to the foot muscles. The anterior compartment such as the tibialis anterior and posterior, popliteus, and extensor digitorum longus appeared better preserved than the posterior compartment. In particular, the MRI changes of the tibialis anterior and posterior were very similar to the EDB, whereas the muscles of the posterior compartment (gastrocnemius and soleus) had advanced changes, mostly grade 3–4. A similar pattern of muscle involvement has been previously reported in T1-weighted studies from patients with DMD with advanced disease.21–23

We finally correlated the extent of histologic muscle pathology, in our case the EDB muscle, with the corresponding muscle MRI involvement. These EDB histopathologic changes correlated well with the MRI changes in 10 of 15 patients in whom EDB biopsies and MRI were available. As increased signal intensity on T1 images may reflect increase in fibrous tissue or in adipose tissue, we also independently scored the presence of adipose tissue in the muscle biopsies, but this did not improve the correlation observed when the extent of the fibrosis was considered. Our correlative histology/imaging studies therefore suggest that MRI grading 2–3 is associated with sufficiently preserved structure, typically grade 2 of histologic changes. Muscle MRI overestimated the structural EDB changes in 2 patients and apparently underestimated them in another 3 patients. This finding might be related to technical difficulties in reproducing and scoring exactly the same section delineation for both MRI and EDB histopathologic studies. One of the limitations is that the EDB bi-
opsy (measuring approximately $2 \times 2 \times 0.5$ cm) needs to be mounted in order to be processed, trimming the border of the sample and orienting the muscle transversally. It is possible that during the trimming process areas of perimysial fat have been removed. Regarding the muscle MRI technique used here, we only used a short protocol with T1 sequences. The use of a longer examination using fat suppression sequences or 3-point Dixon techniques to quantify fibrous tissue might have helped us to obtain further information. However, this would have required a considerably longer examination time and the need for sedation or general anesthesia in several cases, which we did not want to perform. Despite these limitations, in most patients our correlative studies were accurate, which suggests that this muscle MRI protocol has a potential application in assessing the preservation of various muscles without having to verify this invasively.

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DISCLOSURE
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