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Clinical course correlates poorly with muscle pathology in nemaline myopathy

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Abstract—Objective: To report pathologic findings in 124 Australian and North American cases of primary nemaline myopathy. Methods: Results of 164 muscle biopsies from 124 Australian and North American patients with primary nemaline myopathy were reviewed, including biopsies from 19 patients with nemaline myopathy due to α-actin (ACTA1) mutations and three with mutations in α-tropomyosinSLOW (TPM3). For each biopsy rod number per fiber, percentage of fibers with rods, fiber-type distribution of rods, and presence or absence of intranuclear rods were documented. Results: Rods were present in all skeletal muscles and diagnosis was possible at all ages. Most biopsies contained nemaline bodies in more than 50% of fibers, although rods were seen only on electron microscopy in 10 patients. Rod numbers and localization correlated poorly with clinical severity. Frequent findings included internal nuclei and increased fiber size variation, type 1 fiber predominance and atrophy, and altered expression of fiber type specific proteins. Marked sarcomeric disruption, increased glycogen deposition, and intranuclear rods were associated with more severe clinical phenotypes. Serial biopsies showed progressive fiber size variation and increasing numbers of rods with time. Pathologic findings varied widely in families with multiple affected members. Conclusions: Very numerous nemaline bodies, glycogen accumulation, and marked sarcomeric disruption were common in nemaline myopathy associated with mutations in skeletal α-actin. Nemaline myopathy due to mutations in α-tropomyosinSLOW was characterized by preferential rod formation in, and atrophy of, type 1 fibers. Light microscopic features of nemaline myopathy correlate poorly with disease course. Electron microscopy may correlate better with disease severity and genotype.

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Nemaline myopathy (NM) is an uncommon condition characterized by proximal weakness and hypotonia, and defined by the presence in muscle fibers of inclusions known as nemaline bodies or rods (Greek nema = thread). Clinically the disorder has marked clinical variability, with neonatal lethal and mild nonprogressive congenital forms and subtypes with onset in childhood and adulthood.1 NM is genetically heterogeneous. Disease-causing mutations have recently been identified in five different genes, all of which encode protein components of muscle thin filaments: α-tropomyosin (TPM3),2 nebulin (NEB),3 α-actin (ACTA1),4 β-tropomyosin (TPM2),5 and tropo- 
tin T1 (TNNT1).6

NM is a rare disorder in which correlation among clinical course, genotype, and pathologic phenotype is poorly established. Previous pathologic series, which have included no more than 22 cases, have suggested that the number and localization of nemaline bodies may vary widely between patients, between different muscles in a single patient, and even between different parts of the same muscle.7,8 The number and distribution of rods within muscle fibers appears to correlate poorly with age at presentation and clinical severity,9 although the presence of intranuclear rods tends to predict a more severe phenotype.9,10

Other characteristic findings of NM include type 1 fiber predominance and atrophy,8,12 which may become more prominent with increasing age.13 Inflammatory changes, fibrosis, and morphologic changes characteristic of the other congenital myopathies have also been reported in NM but their incidence is uncertain.

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We recently reported clinical findings in 143 patients with NM. Here we describe pathologic findings in 124 of these patients, with the aim of characterizing the relationships between morphologic changes and disease severity, clinical course, and genotype in NM. We also describe pathologic evolution of this disease and discuss the heterogeneity of disease expression in different muscles and within kindreds.

Methods. The diagnosis of NM was based on the European Neuromuscular Centre criteria, which include the pathologic finding of nemaline bodies on muscle biopsy. Patients were ascertained and classified through North American and Australian neurology, genetics, and pathology services as previously described. Informed consent for access to medical records and pathology specimens was obtained from all Australian patients’ physicians and from patients or their parents in all North American cases. The study was approved by the research ethics committees and institutional review boards of all involved hospitals.

Muscle biopsies were processed by, and obtained from, clinical pathology departments and examined after staining with hematoxylin and eosin (H-E), modified Gomori trichrome, and a variety of other standard histochemical methods. These included, in most cases, acid and alkaline phosphatase, adenosine triphosphatase (ATPase) with acid and alkaline preincubation, nonspecific esterase, oil red O, periodic acid Schiff, and NADH-TR. Muscle fibers were classed as type 1 or 2 on serial sections using myosin ATPase staining at pH 4.3 and 9.4.

Atrophy of type 1 and 2 fibers was identified when mean diameters were below normal for age. Type 1 fiber predominance was defined as greater than 55% of fibers and type 2 predominance as >80% of fibers. Internal nuclei were identified when >3% of fibers contained central nuclei on transverse section. Where possible, for each biopsy we documented the percentage of fibers containing rods, the fiber type distribution of rods, and presence or absence of intranuclear rods.

Indirect immunofluorescence was performed using the following antibodies: mouse monoclonal (mAb) antiskeletal slow myosin heavy chain (MHC) (identifies type 1 [slow] fibers) (mAb1628, 1:250, Chemicon International, Temecula, CA, or Novocastra MHC, 1:80, Novocastra, Newcastle, UK); mouse mAb antiskeletal fast MHC (identifies type 2 [fast] fibers) (MY32, 1:200, Sigma, St. Louis, MO, or NCL MHCf, 1:80, Novocastra); mouse mAb antineonatal skeletal MHC (identifies immature and regenerating fibers) (MHC-N, 1:100, Novocastra); affinity-purified rabbit polyclonal anti-α-actinin 2 (1:500), anti-α-actinin 3 (1:50), and β1-syntrophin (1:100, provided by Dr. S. Froehner). AffiniPure goat antimagmouse (1:250) and antirabbit CY3- or fluorescein-conjugated (1:200) secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) and Alexa488-conjugated goat antimouse (1:100, Molecular Probes, Eugene, OR) were used for confocal images. All immunocytochemical studies were repeated on at least two occasions to confirm initial findings and were compared to age-matched control muscle with normal histology.

Stained serial sections were examined under either an Olympus BX50 (Sydney, Australia) or Nikon E1000 microscope (Boston, MA), and photographs recorded with either a SPOT color digital imaging camera or by scanning laser confocal microscopy (Leica SP2, Sydney, Australia). All immunocytochemical studies were repeated on at least two occasions to confirm initial findings and were compared to age-matched control muscle with normal histology.

Figure 1. Biopsy findings in two cases of severe lethal nemaline myopathy (NM). Both infants were ventilator-dependent and died of respiratory insufficiency within the first month of life. (A–C) Skeletal muscle from a 16-day-old boy illustrates several hypoplastic polygonal fibers and fiber size variation on hematoxylin and eosin section (A), cytoplasmic nemaline bodies on Gomori trichrome staining (B), and rods associated with extremely disorganized sarcomeric architecture on electron microscopy (C). (D–F) Quadriceps femoris from a 12-day-old boy. Both epon-embedded (D) and Gomori trichrome–stained (E) preparations show disorganized myofibers and prominent rods with focal loss of striations. Electron microscopy (F) again reveals severe myofibrillar disarray and loss of sarcomeric registration consistent with the severe weakness and hypotonia exhibited by these patients. Scale bars = 20 μm.
For analysis of histochemical and immunocytochemical findings in nemaline myopathy, we used the initial diagnostic biopsy for each patient or, where multiple biopsies were obtained at the same time, findings on biopsy of the quadriceps femoris. These findings were analyzed using Fisher’s exact test. Findings in those undergoing multiple biopsies were analyzed separately.

Subjects. A total of 124 Australian and North American patients with NM underwent 164 muscle biopsies. Where initial biopsies were negative, diagnosis was based on repeat biopsy or postmortem examination of the same or another muscle.

Frozen muscle biopsy specimens or stained slides from 69 patients (92 biopsies in total) were available for review. Electron microscopy (EM) was performed on biopsies from 56 of these patients. Biopsies from 37 of these 69 patients were examined immunocytochemically. Muscle biopsies were unavailable or unsuitable for repeat examination in 55 of the 124 cases (72 biopsies), all of which had been assessed on frozen sections with a range of histochemical stains. Pathologic findings in these biopsies were tabulated as described by the diagnosing neuropathologist. EM reports were available for 32 of these 55 patients.

Of the 124 patients, 105 underwent actin (ACTA1) and 48 α-tropomyosin (TPM3) mutation screening. Information on 23 muscle biopsies was available from 37 of these 69 patients were examined immunocytochemically. Muscle biopsies were unavailable or unsuitable for repeat examination in 55 of the 124 cases (72 biopsies), all of which had been assessed on frozen sections with a range of histochemical stains. Pathologic findings in these biopsies were tabulated as described by the diagnosing neuropathologist. EM reports were available for 32 of these 55 patients.

Results. A wide variety of muscles were studied in life and at postmortem examination. All proved diagnostic in at least one instance. Quadriceps femoris was most frequently sampled (80 biopsies), leading to diagnosis in 88% of cases. The diaphragm, intercostal muscles, psoas, and myocardium were examined only at postmortem examination. Biopsies were diagnostic in all age groups, including 23 children biopsied during the first month of life. Two infants were delivered prematurely had diagnostic quadriceps femoris biopsies at postconceptual ages of 31 and 36 weeks.

No nemaline bodies were seen in 21 biopsies from various muscles. All of these biopsies were studied by frozen section, but only six underwent EM examination. In these patients the diagnosis was made on repeat biopsy of the same or another muscle. Nemaline bodies were seen on trichrome stains in only five of these diagnostic biopsies, and were seen only on EM in 10 patients. The nondiagnostic biopsies were excluded from further analysis.

Nemaline bodies. Rods were rarely seen on H-E stains but were usually apparent on modified Gomori trichrome staining and in epon-embedded thin sections (figures 1 through 4). In 10 biopsies nemaline bodies were identified only on EM. In these cases rods were relatively small, less discrete, and tended to be sarcromeric rather than subsarcolemmal.

In most biopsies (74%), rods were present in more than 50% of extrafusal muscle fibers. Nemaline bodies were variably distributed within myofibers or beneath the sarcolemmal membrane (table 1). Rods were also seen within nuclei in six patients. Both the proportion of rod-containing fibers and rod location (with the exception of intranuclear rods, see below) correlated poorly with disease severity (p values 0.19 and 0.39).

Nemaline bodies were confined to type 1 fibers in 10 of 68 biopsies in which fiber type distribution of rods could be determined (see table 1, figure 3). In eight of these biopsies all rod-

Figure 2. Typical congenital nemaline myopathy (NM), biopsied in infancy. (A, B) Quadriceps femoris, 8 weeks of age. Hematoxylin and eosin staining (A) reveals moderate fiber size variation. Nemaline bodies are not seen. (B) Numerous subsarcolemmal and cytoplasmic rods are apparent on a Gomori-stained section. (C–F) Quadriceps femoris from another case, aged 13 months. Gomori trichrome staining reveals numerous rods (C), which appear as electron-dense bodies interspersed with intact sarcomeres on electron microscopy (D). (E) Myosin ATPase histochemistry at pH 9.4 illustrates marked type 1 fiber predominance with very few darkly staining type 2 fibers (arrows). Indirect immunofluorescence staining (F) for α-actinin 2 (green) and neonatal MHC (red) demonstrates neonatal myosin in a similar (likely identical) population of fibers (arrows). Scale bars = 20 μm.
23 patients whom had type 1 fiber atrophy. Type 2 fiber atrophy was seen in Type 2 fiber predominance was apparent in five patients, four of and 9.4 prevented differentiation between type 1 and 2 fibers. type 1 fiber atrophy/hypotrophy in 44% of cases. In 18 cases inter-


containing fibers were markedly atrophic or hypotrophic. Type 2 fibers in these biopsies were of variable size. The clinical presenta-
tation of patients with these biopsies ranged from severe congeni-
tal to childhood onset NM. Two of these cases were due to the TPM3 M9R mutation, which affects the type 1 fiber-specific iso-
form of α-tropomyosinSlow.2 Another was due to an actin muta-
tion. TPM3 mutations were excluded in five cases, and ACTA1 mutations in six. Rods were never confined to type 2 fibers. Intra-
fusal fibers contained no rods, and fiber distribution was normal
within spindles, even in muscles containing rods in most or all
other fibers.

Fiber typing and size. Fiber-type proportions were abnormal in 70% of biopsies, most commonly because of type 1 fiber predomi-
nance (see table 1, figure 2), which was accompanied by selective type 1 fiber atrophy/hypotrophy in 44% of cases. In 18 cases inter-
mediate staining of all fibers on myosin ATPase stains at pH 4.3 and 9.4 prevented differentiation between type 1 and 2 fibers. Type 2 fiber predominance was apparent in five patients, four of whom had type 1 fiber atrophy. Type 2 fiber atrophy was seen in 23 patients, 8 of whom had ACTA1 mutations (table 2).
A subgroup of biopsies exhibited a unique pattern of findings (see figure 4): 1) subsarcolemmal and cytoplasmic accumulations of well-demarcated rods that stained prominently on modified Go-


Figure 3. Gomori trichrome (A), electron microscopy (B), and immunocyto-
chemical staining for myosin heavy chain (MHC) slow (C), MHC fast (D), α-actinin 2 (E), and α-actinin 3 (F) of sequential muscle biopsy sections from a 46-year-old patient with TPM3 nemaline myopathy. Gomori trichrome staining demonstrates selective rod for-
mation in atrophic/hypotrophic type I fibers (A). Note coexpression of MHC slow and fast in a single fiber (aster-
isk) (C, D). Groups of rods stain positiv-
ely for α-actinin 2 (E), but not for α-actinin 3 (F). Despite the presence of numerous rods, adjacent sarcomeres are well preserved on electron micro-
scopy, consistent with the milder phenot-
type. A, C–F, original magnification ×200.
Several fibers with central nuclei resembling fetal myotubes were present in a single neonate with severe congenital NM. Occasional central cores were reported in six patients, ring fibers in three, and rare minicores in two children. Extramedullary hematopoiesis was identified within muscle in two infants with congenital NM at postconceptual ages 31 to 43 weeks.

EM commonly revealed disorganized sarcomeric architecture with focal or generalized myofibrillar disarray. These findings were most marked in patients with severe clinical presentations (see figure 1) and those with actin mutations.

**Intranuclear rods.** Intranuclear rods were identified in six infants, two of whom were siblings, but in only one of another set of affected siblings. Four had severe congenital NM. Both infants with intermediate congenital NM had antigravity movement and...
Table 1 Rod number, location, fiber size, and typing in nemaline myopathy

<table>
<thead>
<tr>
<th>Finding (no. of biopsies examined for each parameter)</th>
<th>Clinical presentation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Severe congenital</td>
</tr>
<tr>
<td>Patients</td>
<td>23</td>
</tr>
<tr>
<td>Rods &lt;50% fibers (124)</td>
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<td>Rods &gt;50% fibers (124)</td>
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<tr>
<td>Rods seen only on EM (88)</td>
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</tr>
<tr>
<td>Rods primarily sarcomeric (108)</td>
<td>14</td>
</tr>
<tr>
<td>Rods primarily subsarcomeral (108)</td>
<td>5</td>
</tr>
<tr>
<td>Mixed distribution rods (108)</td>
<td>4</td>
</tr>
<tr>
<td>Intranuclear rods (88)</td>
<td>4</td>
</tr>
<tr>
<td>Rods restricted to type 1 fibers (68)</td>
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<tr>
<td>Type 1 fiber predominance (115)</td>
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<tr>
<td>Type 1 fiber hypertrophy (115)</td>
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</tr>
<tr>
<td>Type 1 fiber atrophy/hypotrophy (115)</td>
<td>11</td>
</tr>
<tr>
<td>Type 2 fiber predominance (115)</td>
<td>2</td>
</tr>
<tr>
<td>Type 2 fiber hypertrophy (115)</td>
<td>—</td>
</tr>
<tr>
<td>Type 2 fiber atrophy/hypotrophy (115)</td>
<td>10</td>
</tr>
<tr>
<td>Inability to fiber type on myosin ATPase (115)</td>
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</tr>
<tr>
<td>Increased fiber size variation (117)</td>
<td>17</td>
</tr>
<tr>
<td>Grouped fiber atrophy (124)</td>
<td>—</td>
</tr>
<tr>
<td>Fiber type grouping (124)</td>
<td>1</td>
</tr>
</tbody>
</table>

EM = electron microscopy.

spontaneous respiration at delivery but required ventilatory support from early infancy. One died at the age of 2 months, one survived to 25 months of age.

All biopsies from these infants demonstrated numerous rods in virtually all fibers. Intranuclear rods were present in fewer than 25% of fibers. Increased fiber size variation was not accompanied by type 1 fiber predominance or atrophy. EM revealed marked sarcomeric disorganization in most fibers. Degenerating fibers and increased fibrosis were identified in single cases. In one patient (reported as Patient 1 in a previous series21), a subset of sarcomeres were dramatically atrophied fibers was apparent on EM. Immuno-
cytocytochemical analysis of a single case revealed coexpression of fast and slow MHC and of slow MHC and α-actinin 3.

Two of these infants had ACTA1-linked NM. Pathologic find-
ings in their biopsies did not differ from the other four cases, two of whom had no identifiable mutation. Two infants were not genotyped for actin mutations.

Multiple biopsies. Thirty patients underwent multiple biopsies. In 18 cases two or more muscles were sampled at the same time in life or as part of postmortem examination. In eight of these patients (aged 3 days to 16 years at time of biopsy), all muscles showed similar histopathologic abnormalities. In 10 pa-
tients (aged 7 days to 46 years), changes in truncal muscles were more marked than those in the extremities. Rod-containing fibers were more numerous in the diaphragm and intercostal muscles than in appendicular muscles in six of seven ventilator-dependent infants. Pathologic changes were generally more marked in the quadriceps femoris than biceps brachii, triceps brachii, or deltoid. In one case of childhood onset NM investigated at 17 years of age, the biceps brachii biopsy results were normal (with no rods and normal fiber typing), whereas the quadriceps femoris biopsy demon-
strated type 1 fiber predominance and rods in virtually every muscle fiber.

Twelve patients underwent serial biopsies at intervals of 7 months to 32 years. Repeat biopsy was of the same muscle in three patients and a different muscle in four cases. In five cases biopsied muscles were not specified. Both the percentage of fibers with rods and the degree of fiber size variation increased with time in seven cases. Progressive muscle fibrosis was seen in five patients. Progressive type 1 fiber predominance was seen in three patients, and increasing type 2 fiber predominance in one case.

Initial muscle biopsy was nondiagnostic in five cases. Four children aged 2 months to 13 years had biopsies showing only fiber type disproportion. Repeat biopsy of the same or a different muscle group after intervals as long as 19 years revealed numerous nemaline bodies in all patients. A patient with adult onset NM had unremarkable quadriceps femoris biopsies at 44 and 47 years of age before diagnostic deltoid biopsy at 50 years.

Biopsy of multiple family members. Of eight phenotypically homogeneous families, similar biopsy findings (fiber type, percentage fibers with rods, and rod distribution) were seen in five kin-
dreds (one of which had a TPM3 mutation), the other three demonstrating disparate pathologic abnormalities. Two families with varied clinical phenotypes, one of which carried an ACTA1 mutation, had heterogeneous biopsy findings. Pathologic abnor-
malities in these kindreds did not correlate with disease severity.

Three patients had mildly abnormal biopsy results (with rods in less than 5% of fibers), which were believed to be nondiagnostic until other family members were investigated.

Nemaline bodies were identified on muscle biopsy in two of six clinically unaffected parents. In one case both parents had abnor-
mal biopsies, one containing several atrophic fibers with rods, the other showing excessive Z-line streaming. It was unclear whether the parents were manifesting heterozygotes or one had subclinical

dominant NM.

Myocardial pathology. Nemaline cardiomyopathy was present in two siblings with severe congenital NM who died at 1 day and 4 weeks of age. Neither had clinical evidence of cardiac involvement. In both cases myocardial examination revealed nemaline bodies and mild disorganization of cardiac sarcomeric structures, with normal intercalated discs. Intranuclear rods were present within the myocardium of one child and the skeletal mus-
Table 2 Rod number, location, fiber size, and typing in ACTA1/TPM3-linked nemaline myopathy (NM)

<table>
<thead>
<tr>
<th>Finding (no. of ACTA1/TPM3 NM biopsies examined for each parameter)</th>
<th>ACTA1 NM</th>
<th>TPM3 NM</th>
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<td>Patients (6/3/1)</td>
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<td>Intermediate congenital</td>
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<tr>
<td>Rods &lt;50% fibers (19/3)</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Rods &gt;50% fibers (19/3)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Rods seen only on EM (16/2)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Rods primarily sarcomeric (19/3)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Rods primarily subsarcolemmal (19/3)</td>
<td>3</td>
<td>1</td>
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<tr>
<td>Mixed distribution rods (19/3)</td>
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<tr>
<td>Intranuclear rods (19/3)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rods restricted to type 1 fibers (12/3)</td>
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<td>Increased fiber size variation (19/3)</td>
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<td>Fiber type grouping (19/3)</td>
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EM = electron microscopy.

Discussion. Characteristic pathologic changes in NM may be expressed in all skeletal muscles and at all ages. Muscle biopsy is likely to be diagnostic even during the neonatal period. In this series, as in previous reports, the number and distribution of nemaline bodies generally correlated poorly with age at presentation and severity of NM.6,13 Numbers of rod-containing fibers varied widely between patients and even between different muscles in individual cases.7 Increased rod numbers in the respiratory muscles may correlate with ventilatory insufficiency.7,8 In three patients with actin mutations underwent multiple biopsies. Little pathologic evolution was seen on repeat biopsy after as long as 22 years.

Alpha troponemin mutations. NM due to TPM3 mutations was characterized by markedly increased fiber size variation and internal nuclei (see table 2). Nemaline bodies were present only within type 1 fibers in two patients, in whom marked type 1 atrophy and predominance was accompanied by type 2 fiber hypertrophy (see figure 3). Rods were present in both fiber types in the third patient, a surprising finding possibly related to coexpression of fiber-specific proteins. Nemaline bodies were generally less numerous in cases with TPM3 mutations than in those with actin mutations. Serial biopsy revealed increased fiber size variation, numbers of rod-containing fibers, and endomysial connective tissue in two cases. In two patients studied immunohistochemically, a minority of fibers coexpressed slow and fast MHC isoforms and α-actinin 3 (see figure 3). Uncoupling of α-actinin 3 and β1-syntrophin or fast MHC expression was seen in 50% of type 2 fibers. Expression of α-actinin 2 was normal.

EM = electron microscopy.

NM in patients with known genetic mutations. Actin mutations. Percentages of rod-containing fibers and rod size varied widely in patients with actin mutations and showed some correlation with disease severity (see table 2). A total of 84% of all patients with ACTA1 mutations had rods in 50 to 100% of fibers, as compared to 43% of patients with NM unrelated to actin mutations (p = 0.002). Rods were present in both type 1 and 2 fibers in all but one biopsy. Two patients had intranuclear rods.

Light microscopy in these cases commonly revealed increased fiber size variation and internal nuclei. Type 1 fiber predominance was seen in 67% of biopsies, and atrophy in 39%. Type 2 fiber atrophy was seen in 8 biopsies (44%), and was more common in this than in NM unrelated to ACTA1 mutations (p = 0.03). Perimysial connective tissue was increased in four cases. Findings in single biopsies included collections of whorled thin filaments within fibers and occasional central cores.

EM in cases with actin mutations frequently revealed marked disruption of the sarcomere register, in association with accumulations of thin filaments in most fibers. These abnormalities were most marked in patients with severe congenital NM and those with large numbers of rods. Dramatically atrophied fibers were seen on EM, but not light microscopy, in two biopsies, one of which also included intranuclear rods, and three biopsies contained populations of degenerating cells. Increased sarcoplasmic and intermyofibrillar glycogen stores were present in 11 patients. In two cases increased glycogen stores not apparent on periodic acid Schiff stains were clearly evident on EM.

Immunohistochemical staining revealed coexpression of fast and slow MHC isoforms in up to 50% of fibers in three of eight patients. Abnormal α-actinin 3 expression was identified in type 1 fibers in five biopsies.
this study these muscles were biopsied only at autopsy, and their apparently more severe involvement may therefore reflect an ascertainment bias. Rod location within fibers or the subsarcolemmal region did not correlate with disease severity or course. Intranuclear rods, however, as previously reported, were associated with early presentation and severe weakness.3,11

Fiber size variation most frequently reflected type 1 fiber atrophy. Type 1 fiber predominance has been suggested as a primary cause of muscle weakness in NM,18 but in this series abnormalities of fiber typing and size generally correlated poorly with clinical classification and course.

Abnormal fiber typing in NM has been attributed to selective type 1 fiber formation,8,18,19 delayed maturation of fiber typing,19 or to fiber type conversion.20 Type 1 fiber predominance may represent a secondary, compensatory change, slow fibers having more energy-efficient oxidative metabolism.19,20 In this series, myosin ATPase studies failed to differentiate type 1 and 2 fibers in 18 biopsies, although immunocytochemical studies in these patients demonstrated expression of at least one protein specific to slow muscle fibers. Abnormal expression of MHC isoforms and other structural proteins within type 1 and type 2 fiber populations was seen on immunocytochemical studies in all subtypes of NM. These findings suggest either active fiber type conversion or delayed muscle maturation in all clinical variants of this condition.

Findings such as internal nuclei, fibrous infiltration, and inflammatory or degenerative changes within muscle correlate neither with age at onset nor disease severity in NM. The presence of occasional morphologic abnormalities suggestive of other congenital myopathies in patients with NM probably reflects common secondary pathologic changes in response to varied primary insults.7,21–23 Two patients in this series had extramedullary hematopoiesis within muscle, a finding not previously described in NM but which has been suggested to reflect delayed muscle maturation when seen in patients with congenital fiber type disproportion.24

Pathologic evolution on serial biopsy was difficult to interpret in cases where different muscles were biopsied. In such cases apparent disease progression might actually reflect variable disease expression. However, there were trends to increased numbers of fibers with rods, fiber size variation, and muscle fibrosis with increasing age.13 Five patients were diagnosed only on repeat biopsy, possibly because of pathologic evolution with increasing age13,22 or sampling error.

Two characteristic pathologic phenotypes were identified in this study: a subset of patients with intermediate staining of uniformly sized muscle fibers and large numbers of rods in most fibers, and another subset with marked type 1 fiber prominence and atrophy in association with selective rod formation within type 1 fibers. The association of the former variant with typical, nonprogressive NM, and concordant findings in two affected siblings, are consistent with the hypothesis that this pathologic phenotype may be genetically determined. Two patients with the second variant had TPM3 NM, with onset of primarily distal weakness in adolescence, but others with similar pathologic findings had severe weakness from the first year of life and no demonstrable mutation in TPM3.

This study demonstrates the importance of EM in muscle biopsy evaluation. In 10 patients NM would not have been diagnosed had EM not been performed, and the diagnosis was likely missed in a subset of patients whose initial biopsies were not subjected to EM examination. EM also facilitates the identification of intranuclear rods and glycogen accumulation within muscle. In this series, accumulation of myofilaments and loss of sarcomeric registration were common in patients with severe weakness, including those with intranuclear rods and those with ACTA1 mutations. The extent of disruption of sarcomeric structures on EM appears to correlate better with clinical severity of NM than do light microscopic abnormalities.

Ultrastructural changes on EM—diffuse sarcomeric disruption and glycogen accumulation—are suggestive of (but not specific for) NM due to actin mutations, which on histopathologic examination may also be suggested by the finding of type 2 fiber atrophy. TPM3 mutations may be associated with marked fiber size variation and production of two distinct populations of fibers—numerically predominant atrophic type 1 fibers and normal or hypertrophied type 2 fibers—and with preferential rod formation in type 1 fibers. None of these features was entirely specific to this form of NM. Improved detection of causative mutations in other genes, particularly nebulin, will be required for more comprehensive evaluation of genotype–phenotype correlation in NM.

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