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Retrospective Cohort Analysis of Clinical, Molecular, and Histopathologic Characteristics of 275 Patients With Nemaline Myopathy

Clara Hildebrandt,[1] Casie A. Genetti,[1] Tanya Logvinenko,[2] Wathone Win,[1] Pamela Barraza-Flores,[1] Leslie H. Hayes,[3] Shira Rockowitz,[1,4] Vilma-Lotta Lehtokari,[5] Susan T. Iannaccone,[6] Basil T. Darras,[7] Haluk Topaloglu,[8,9] Carina Wallgren-Pettersson,[5] and Alan H. Beggs[1,10]

Correspondence
Dr. Beggs
beggs@enders.tch.harvard.edu

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Abstract

Background and Objectives

Nemaline myopathy (NM) is a congenital myopathy with a wide severity spectrum, from severe, generalized muscle weakness and respiratory failure in the neonatal period to mild, distal weakness in young adulthood. Eleven genes have been definitively established to cause the condition. Although some recurrent variants have been identified, the overall correlation of genotype with clinical severity in NM remains poor. This poses challenges when counseling families about prognosis after a diagnosis of NM is made. Better clinical and molecular predictors of outcome are needed for clinical trial readiness.

Methods

A retrospective cohort analysis of 275 patients with a histopathologic diagnosis of NM and/or pathogenic variants in NM-associated genes was performed to identify relationships between early clinical findings and long-term outcomes, including need for respiratory and feeding support.

Results

Early clinical predictors of long-term outcomes were identified: patients with hypotonia at birth had increased odds of requiring gastrostomy tubes, and patients with respiratory distress at birth had increased odds of requiring both gastrostomy tubes and invasive ventilation. Individuals with *ACTA1*-NM were more likely to require feeding tubes and invasive ventilation in the first year of life compared with those with *NEB*-related NM, but the odds of requiring invasive ventilation were similar after the first year of age. Additional clinical information is presented by genotype and severity class.

Discussion

Neonatal findings of individuals with NM are correlated with long-term clinical outcomes, and there are some relationships between genotype and disease severity. Prospective longitudinal studies are needed to confirm these findings and evaluate for additional early clinical predictors of prognosis.

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Supplementary Material

Introduction

Nemaline myopathy (NM) is a nonprogressive or slowly progressive congenital myopathy first defined by the presence of so-called nemaline rods over a half century ago.[1,2] At the severe end of the spectrum, infants can present with fractures and contractures at birth and may never gain

[1]Division of Genetics and Genomics, The Manton Center for Orphan Disease Research, Boston Children's Hospital, Harvard Medical School, MA; [2]Clinical Research Center, Boston Children's Hospital, Harvard Medical School, MA; [3]Department of Neurology, The Manton Center for Orphan Disease Research, Boston Children's Hospital, Harvard Medical School, MA; [4]Children's Rare Disease Collaborative, Boston Children's Hospital, MA; [5]Folkhälsan Research Center and Department of Medical Genetics, Medicum, Faculty of Medicine, University of Helsinki, Norway; [6]Departments of Pediatrics and Neurology, University of Texas Southwestern Medical Center, Dallas, TX; [7]Department of Neurology, Boston Children's Hospital, Harvard Medical School, MA; [8]Department of Pediatric Neurology, Hacettepe University Children's Hospital, Ankara, Türkiye; [9]Department of Pediatrics, Yeditepe University, İstanbul, Türkiye; and [10]The Broad Institute of Harvard and MIT, Cambridge, MA.

Glossary

CK = creatine kinase; **GT** = gastrostomy tube; **NM** = nemaline myopathy; **ΔExon55** = exonic deletion.

respiratory independence or substantial antigravity movements. Individuals with a milder clinical picture experience motor delays but may have normal respiratory function. Bulbar weakness affecting speech and swallowing function is variable. Diagnostic criteria were initially established by a European Neuromuscular Centre workshop in 1998.[3] Diagnosis is based on histopathologic findings of nemaline rods on muscle biopsy, clinical features of myopathy, and molecular testing of genes implicated in NM.

Nemaline rods are focal collections of Z-disc–derived proteins noted on light microscopy of Gomori trichrome–stained muscle and are readily apparent on electron microscopy as dark, electron dense collections near Z-discs. Other characteristic histopathologic findings include fiber size variation and type 1 fiber predominance.[4] According to ClinGen, there is moderate or definitive evidence that pathogenic variants in 11 genes may cause NM: *ACTA1, CFL2, KBTBD13, KLHL40, KLHL41, LMOD3, MYPN, NEB, TNNT1, TPM2, TPM3*.[5,6] The final common pathogenic pathway is dysregulation of muscle thin filaments and thereby cross-bridge cycling.[7–10] There is also evidence for myopathy with NM features in some patients with pathogenic variants in *TNNT3, ADSS1, MYO18B, CAP2, RYR1*, and *RYR3*,[11–16] although none of these has been classified as having moderate or definitive evidence for NM.[6]

Diagnosis can be complicated by differences in histopathologic findings between different muscle samples from the same patient, or changes over time if a repeat biopsy is obtained. Molecular testing to identify the genetic basis is critical because of the extensive genetic heterogeneity as well as clinical and pathologic variability, including the absence of rods in some biopsies and patients. Predicting prognosis in patients with newly diagnosed NM is difficult because few reproducible genotype-phenotype correlations have been established.[17] This is exemplified by the finding of marked variation in severity even within the same family.[18,19] A clinical classification ("2000 NM Classification Scheme") was initially proposed in 2000 (eTable 1) to categorize the broad spectrum of clinical severity.[20] In addition to severe, intermediate, typical, and mild classes categorized by motor symptoms and respiratory impact, 2 additional classifications included a rapidly progressive adult-onset class and an "other" category with uncommon findings (ophthalmoplegia, cardiomyopathy, unusual distribution of weakness, intranuclear rods). Adult-onset NM remains incompletely understood but likely represents a distinct condition with unique pathophysiology that appears to involve immune or inflammatory dysregulation and may not be a monogenic condition.[21,22] A revised classification was proposed in 2019 ("Sewry NM Classification Scheme") that eliminated the "intermediate" and "other" categories but conflated genotypes with phenotypes into 1 scheme with several gene-specific categories and several clinical ones[23] (eTable 1). After a further revision in 2021,[24] a numerical severity scoring system ("Amburgey score") incorporating ambulation, respiratory support, and feeding support was proposed based on a prospective assessment of 57 individuals with *NEB, ACTA1*, or *TPM2* pathogenic variants or histopathologic diagnosis of NM.[25] Nevertheless, characterization of multiple cohorts with each of these classifications or scoring schemes has not yielded reliable predictors of long-term outcomes in NM.

Genotype-phenotype correlation is important to provide families with an estimate of prognosis in the newborn period when critical decisions about level of respiratory support are made. When genotype is an insufficient predictor of long-term outcome, other information available in the first few months of life should be assessed for predictive power. A comprehensive understanding of the natural history of NM and a reliable way to predict disease severity are crucial in designing clinical trials for this condition. To assess the relative values of clinicopathologic and genetic diagnoses, this study reviews a large cohort of patients with clinicopathologic diagnoses of NM and/or pathogenic variants in NM-related genes. We describe and quantify common clinical characteristics and complications of individuals with NM by genotype and severity. Analysis of this cohort reveals that certain genotypes, as well as early clinical findings, are associated with increased medical interventions, including need for respiratory and feeding support.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the Institutional Review Board of Boston Children's Hospital (IRB protocol 03-08-128R). Inclusion criteria required either a clinicopathologic or molecular diagnosis of NM with pathogenic variants in one of the 11 genes with moderate or definitive evidence for NM disease association as determined by the ClinGen Congenital Myopathies Gene Curation Expert Panel.[6] A positive clinicopathologic diagnosis is defined as a clinical history/examination consistent with congenital myopathy and muscle biopsy diagnostic for NM in that individual or a similarly affected relative. Thirty-two individuals had pathologic diagnosis other than NM and were included on the basis of having mutations in one of the recognized NM genes (eTables 2–4). Patients were ascertained by physician or self-referral from multiple centers and not independently

examined by study clinicians. Ascertainment biases are discussed in limitations. Patients were consented and enrolled from 1990 to 2019, and clinical data were collected from referring physicians, by medical record release procedures, and/or from the patients/families themselves. Paper charts and electronic research database records of eligible patients were reviewed. Where necessary and possible, patients were recontacted to provide missing information and clinical data were updated over time when available. Data across 5 major categories were collected: demographic information, medical history, neurologic assessments, histopathology, and molecular testing. Three hundred forty-seven charts were reviewed, and 275 contained sufficient information for analysis and were thus included in the study. Individually identifiable information on 97 patients was previously included in other published studies (eTables 2–4).[1,4,7,8,17,19,21,25–55]

Statistical Analysis

Statistical analyses, software packages, methods, and models are summarized in eTable 5. For each analysis, all cases with available information were included (no exclusion of outliers).

Qualitative data included medical history, developmental history, examination findings, and histology findings, and the data dictionary used to encode these for statistical analysis is provided as an eAppendix. Motor development was defined as delayed if independent ambulation was achieved after 14 months of age. Patients who died before 1.5 years of age were excluded from the analysis of motor and ambulation status. Patients were classified using the 2000 NM Classification Scheme,[20] as well as the updated Sewry NM Classification Scheme.[23] The mean age at last clinical evaluation was 14.6 years (range birth to 76 years). Six patients were excluded from certain outcome analyses because either the pregnancy was terminated or the child was stillborn or died within hours of birth. These cases were included in comparisons of genotype with clinical severity because all 6 had severe features identified on prenatal imaging or birth examination.

Molecular Testing

Two hundred thirty-nine patients had clinical or research-based genetic testing while 36 had no testing reported. Variants underwent variant interpretation according to the American College of Medical Genetics 2015 guidelines,[56] supplemented by reports in ClinVar,[57] where available, and unpublished functional and other data are provided in eTables 2–4. Given the difficulty in identifying *NEB* variants because of complexity and size of the gene, patients with clinicopathologic diagnoses of NM and only 1 identifiable pathogenic or likely pathogenic variant in the *NEB* gene, with no evidence of disease-causing variants in other known NM genes, were classified as cases of *NEB*-NM for purposes of subsequent analysis.

Data Availability

Deidentified data not published within this article will be made available on request from any qualified investigator.

Results

Cohort Characteristics

Two hundred seventy-five patients, representing 233 unrelated kindreds, met inclusion criteria for this study (Table 1). Of these, 70 had a clinicopathologic diagnosis of NM without molecular confirmation of a genetic cause, because either they did not undergo genetic testing or genetic testing was nondiagnostic, 171 had a clinicopathologic and molecular diagnosis, and 7 had a molecular diagnosis without having undergone a muscle biopsy themselves or in any affected family member. Thirty-two individuals had likely pathogenic or pathogenic variants in an NM gene, but other pathologic diagnoses, including congenital fiber-type disproportion (20), cardiomyopathy (3), multiminicore disease (2), cap myopathy (3), and congenital myopathy with nonspecific histologic findings (4). Ninety-five individuals had *NEB*-NM, 79 had *ACTA1*-NM, 18 had *TPM3*-NM, 9 had *TPM2*-NM, 5 had *LMOD3*-NM, 7 had *TNNT1*-NM, 3 had *KLHL40*-NM, 2 had *KLHL41*-NM, and 2 had *CFL2*-NM (eTables 2–4). Sixty-three were classified as severe congenital, 59 as intermediate, 117 as typical congenital, 24 as mild/juvenile onset, and 12 as adult onset (eTable 6). Two patients with milder symptoms who fit best in the typical/congenital category did have contractures at birth (pseudocamptodactyly, talipes equinovarus, and finger contractures), and 1 had a fracture at birth (clavicle).[51,58] Scoliosis was seen in each severity class except for the adult-onset group. Fifty-six had atypical features; 6 of 236 patients with relevant information had ophthalmoplegia reported in the medical record, 36 of 219 had unusual distribution of weakness, 12 of 226 had cardiomyopathy, and 7 patients had intranuclear rods. Atypical genetic findings included 2 patients with autosomal recessive *ACTA1* loss-of-function variants, 5 with autosomal recessive *TPM3*-NM, and 1 with de novo dominant *NEB* mutation presenting as congenital fiber type disproportion (Pt. 1304-1, eTables 3 and 7). Recessive cases of *ACTA1*-NM were invariably severe. In addition, parental mosaicism was questioned in 2 families with *ACTA1*-NM, but mosaicism studies were not available on these parents. One hundred fourteen patients (42% of the total cohort) had a family member with NM. There were 19 individuals born from consanguineous unions (Table 1). eTable 8 provides the total number of patients with variants in each gene and selected clinical characteristics.

Age at Onset and Age at Diagnosis

The age at symptom onset and age at diagnosis were ascertained by genotype and severity class for patients with available information (Figure 1). The median age at onset was similar for patients with the most common molecular causes of NM: *NEB, ACTA1, TPM2,* and *TPM3*. The median at diagnosis was lowest for *ACTA1, KLHL40,* and *LMOD3* groups. The median age at symptom onset was similar for patients with severe, intermediate, and typical/congenital NM, but the severity of these symptoms varied. Except for 1 patient with adult-onset *TNNT1*-NM with a dominant pathogenic variant, all patients with variants in *TNNT1, KLHL40, KLHL41, LMOD3,* and

Table 1 Overall Cohort Characteristics

Total patients included	275
Ancestry, n (%)[a]	
African American	15 (6)
Ashkenazi Jewish	24 (8)
Asian	16 (6)
European	193 (70)
Hispanic	39 (14)
Middle Eastern	13 (5)
Native American	13 (5)
Other	6 (2)
Unknown	23 (8)
More than 1 ethnicity	64 (24)
Consanguinity, n (%)[b]	
Yes	19 (7)
Sex, n (%)	
M	131 (48)
F	144 (52)
Family history, n (%)[c]	
No	143 (52)
Yes	114 (42)
Unknown	18 (6)
Status, n (%)	
Living	209 (75)
Deceased	61 (22)
Unknown/NA	5 (2)
Birth, y (mean, median, range)[d]	269 (1999, 2005, 1939–2017)
Age in 2021 for all living patients, n (mean, median)	205 (37.3, 32)
Age at last evaluation,[e] n (mean, median)	267 (14.7, 8)
Clinicopathologic diagnosis, n (%)	
Nemaline myopathy	244 (89)
Cap myopathy	2 (1)
Cardiomyopathy	3 (1)
Congenital myopathy NOS	4 (1)
Congenital fiber-type disproportion	20 (7)
Multiminicore disease	2 (1)
Method of ascertainment, n (%)	
Neurology clinic/consult	130 (47)
NICU	75 (27)
Affected family member	31 (11)
Other	10 (4)
Unknown	29 (11)

Abbreviation: NA = not applicable; NICU = neonatal intensive care unit; NM = nemaline myopathy; NOS = not otherwise specified.
[a] Self-reported identities. Several individuals had multiple ancestries; therefore, individual percentages do not add to 100.
[b] Information on consanguinity was not available for 141 cases.
[c] Family history of diagnosed NM or family member with consistent symptoms.
[d] Includes 3 terminations.
[e] Either clinical examination or study questionnaire.

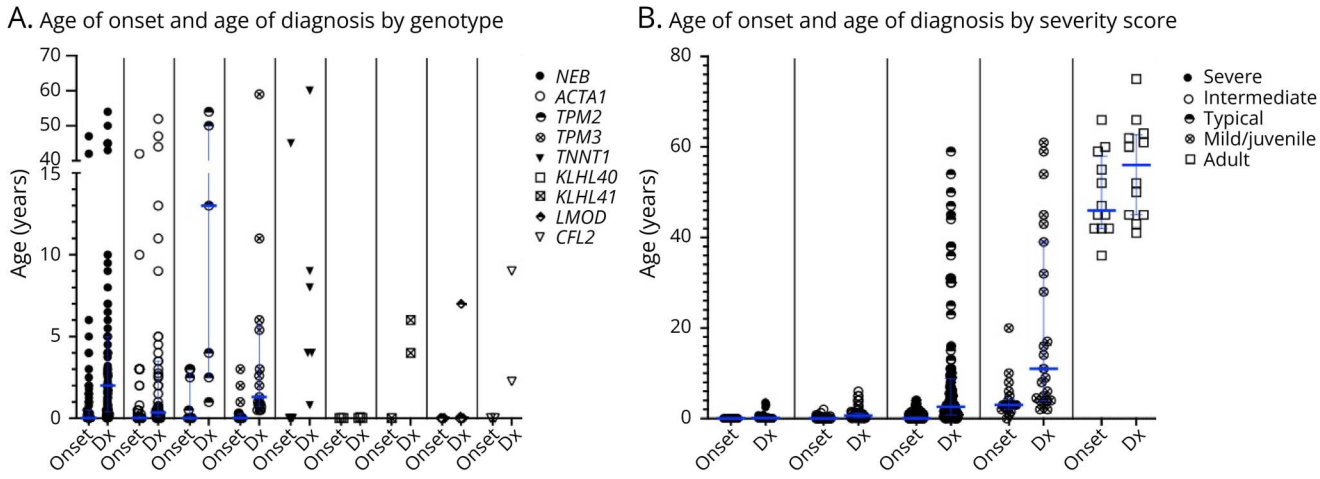
CFL2 had congenital onset. Patients with *KLHL40*-NM and *LMOD3*-NM were generally diagnosed shortly after birth. Cases of *TNNT1*-NM, *KLHL41*-NM, and *CFL2*-NM often had delayed diagnosis despite onset in the neonatal period.

Survival and Loss of Ambulation

Time to death and time to loss of ambulation were evaluated using Spearman correlation. Two hundred sixty-three patients were live-born and had sufficient information available for inclusion in survival analysis. Among 61 patients who died, the mean age at death was 6.1 years and the median age at death was 0.2 years. Of 8 individuals who had information available past 65 years, 2 individuals died at age 66 and 6 lived past age 66 (Figure 2A). Survival between genotype groups with enough informative cases (*NEB*-NM, *ACTA1*-NM, and *TPM3*-NM groups and those without a molecular diagnosis) was compared (Figure 2B). Survival durations differed between the 4 groups of patients ($p = 0.02$). Although sample sizes were too small for meaningful comparisons, no patients with *TPM2*-NM died (age at last evaluation 7–58 years), 1 patient with *TNNT1*-NM died at 66 years, 2 patients with *KLHL40*-NM died in infancy, neither of the patients with *KLHL41*-NM died (follow-up ages 16 and 17 years), 3 patients with *LMOD3*-NM died in infancy, and neither of the patients with *CFL2*-NM died (follow-up ages 2.25 and 9 years).

Comparison of survival between individuals requiring invasive ventilation in the first year of life and those with positive pressure support (noninvasive ventilation), and those without respiratory support, showed different survival durations between the 3 groups of patients ($p < 0.001$), with patients requiring no respiratory assistance surviving longer. The median survival time in the group with invasive ventilation was 0.75 years, with 31 of 51 patients dying. Median survival time in patients with no respiratory assistance up to 1 year of age and those with positive pressure ventilation cannot be assessed because only 9 of 134 patients died in the no-support group and 2 of 11 died in the positive pressure group (Figure 2C). Survival durations were compared between patients with respiratory failure and those without this finding,

Figure 1 Ages at Onset and Diagnosis by Genotype and Clinical Severity Scores



Age at onset and age at diagnosis by genotype (A) and 2000 NM Classification Scheme (B). The vertical lines represent the interquartile range, and the horizontal line represents the median. (A) The median age at onset was 0.01 years for patients with *NEB*-NM (n = 95, IQR 0.01–0.5), *ACTA1*-NM (n = 77, IQR 0.01–0.07), *TPM2*-NM (n = 9, IQR 0.01–2.7), and *TPM3*-NM (n = 17, IQR 0.01–0.28). The median age at diagnosis for patients with *NEB*-NM was 2.0 years (n = 92, IQR 0.42–5.0), *ACTA1*-NM was 0.35 years (n = 74, IQR 0.1–3.5), *TPM2*-NM was 13.0 years (n = 7, IQR 2.5–50.0), and *TPM3*-NM was 1.3 years (n = 17, IQR 0.75–5.7). (B) The median age at onset was 0.01 years for patients with severe NM (n = 60, IQR 0.01–0.01), intermediate NM (n = 58, IQR 0.01–0.01), and typical/congenital NM (n = 112, IQR 0.01–0.25); 3.0 years for those with mild/juvenile NM (n = 22, IQR 2.0–4.25); and 46.0 years for those with adult-onset NM (n = 12, IQR 42–58). The median age at diagnosis for patients with severe NM was 0.08 years (n = 59, IQR 0.04–0.2), intermediate NM was 0.6 years (n = 57, IQR 0.76–8.75), typical/congenital NM was 2.6 years (n = 104, IQR 0.76–8.75), mild/juvenile NM was 11.0 years (n = 23, IQR 4.0–39.0), and adult-onset NM was 56.0 years (n = 12, IQR 45–62.8). IQR = interquartile range.

showing a difference between the 2 groups of patients ($p < 0.001$), with patients with no respiratory failure surviving longer. The median survival time in patients with respiratory failure was 0.68 years; for patients without respiratory failure, it could not be assessed because only 26 of 209 patients died (Figure 2D).

Analysis of loss of ambulation (either complete or partial) was based on 212 individuals who survived or were followed up after age 1.5 years with known ambulation status. Sixty of them were nonambulant (average age at last evaluation 23 years), 17 required occasional use of a wheelchair or other mobility assistance, and 129 were ambulatory. Median time to complete or partial loss of ambulation in patients able to walk cannot be assessed because only 25 of 153 patients who were previously ambulatory lost ambulation (Figure 2E).

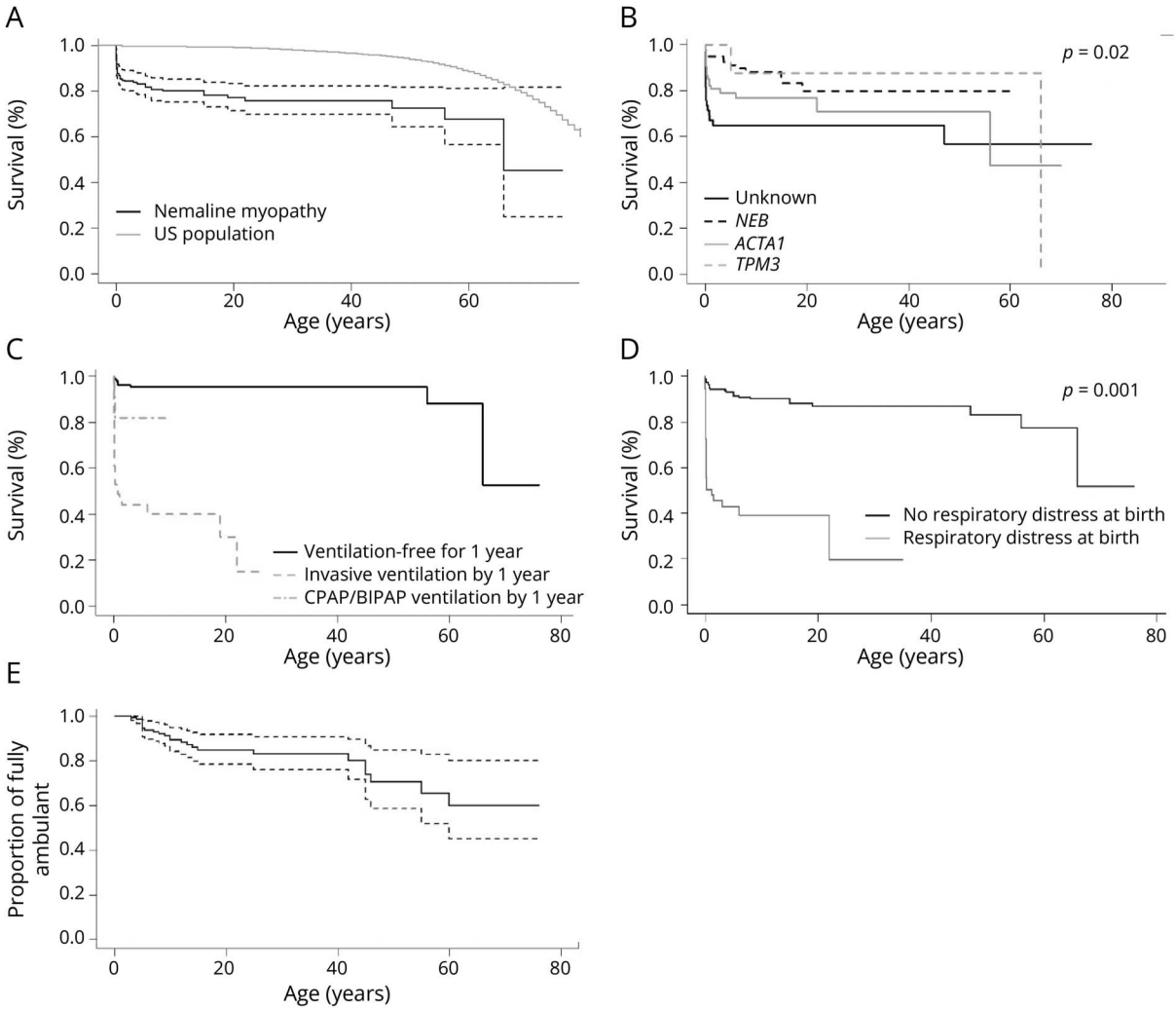
Odds Ratios

Odds ratios were used to investigate relationships between clinical findings (hypotonia at birth, respiratory distress at birth) and long-term outcomes (Figure 3A), as well as genotype and clinical findings (Figure 3B) and birth examination findings and genotype (Figure 3C).

Patients whose medical records indicated hypotonia at birth were more likely to require a gastrostomy tube (GT) (OR = 2.2, 95% CI 1.3–3.7) than patients without hypotonia at birth but were not more likely to require invasive ventilation in the first year of life or later in life. Patients whose medical records indicated respiratory distress at birth compared with those without this finding were more likely to require a GT (OR = 4.8, 95% CI 2.6–8.6), invasive ventilation in the first year of life (OR = 2.8, 95% CI 1.5–5.5), and invasive ventilation after the first year (OR = 3.3, 95% CI 1.8–6.1). Of 105 patients who required a GT, 21 eventually had the GT removed. Of the patients who required GT placement, there was no difference in odds of later removal between those with hypotonia and those with no hypotonia at birth, or between patients with respiratory distress and those with no respiratory distress at birth (Figure 3A).

Genotypic analysis reveals that severe congenital NM was more likely in patients with *ACTA1*-NM compared with patients with *NEB*-NM (OR = 5.4, 95% CI 2.31–12.11). Patients with *ACTA1*-NM were more likely to require invasive ventilation in the first year of life compared with patients with *NEB*-NM (OR = 4.7, 95% CI 1.9–11.0), but not later in life, and were more likely to require a GT (OR = 2.2, 95% CI 1.2–3.9). Patients with *NEB*-NM vs *ACTA1*-NM did not appear to have different odds of presenting with NM symptoms at birth, but patients with *NEB*-NM and the common exon 55 deletion were more likely to be symptomatic at birth than patients with other variants in *NEB* (OR = 7.15, 95% CI 1.2–78.9). In patients with a GT, there was no difference in odds of GT removal between patients with *NEB*-NM and those with *ACTA1*-NM (Figure 3B). Patients presenting with hypotonia at birth did not have higher odds of having pathogenic *NEB* vs *ACTA1* variants identified. Similarly, patients with respiratory distress at birth did not have higher odds of having pathogenic *NEB* vs *ACTA1* variants identified, and there was also no difference in odds of identifying pathogenic *TPM2* vs *TPM3* variants (Figure 3C).

Figure 2 Survival and Time-to-Event Analyses of Censored Data by Diagnosis and Clinical Findings

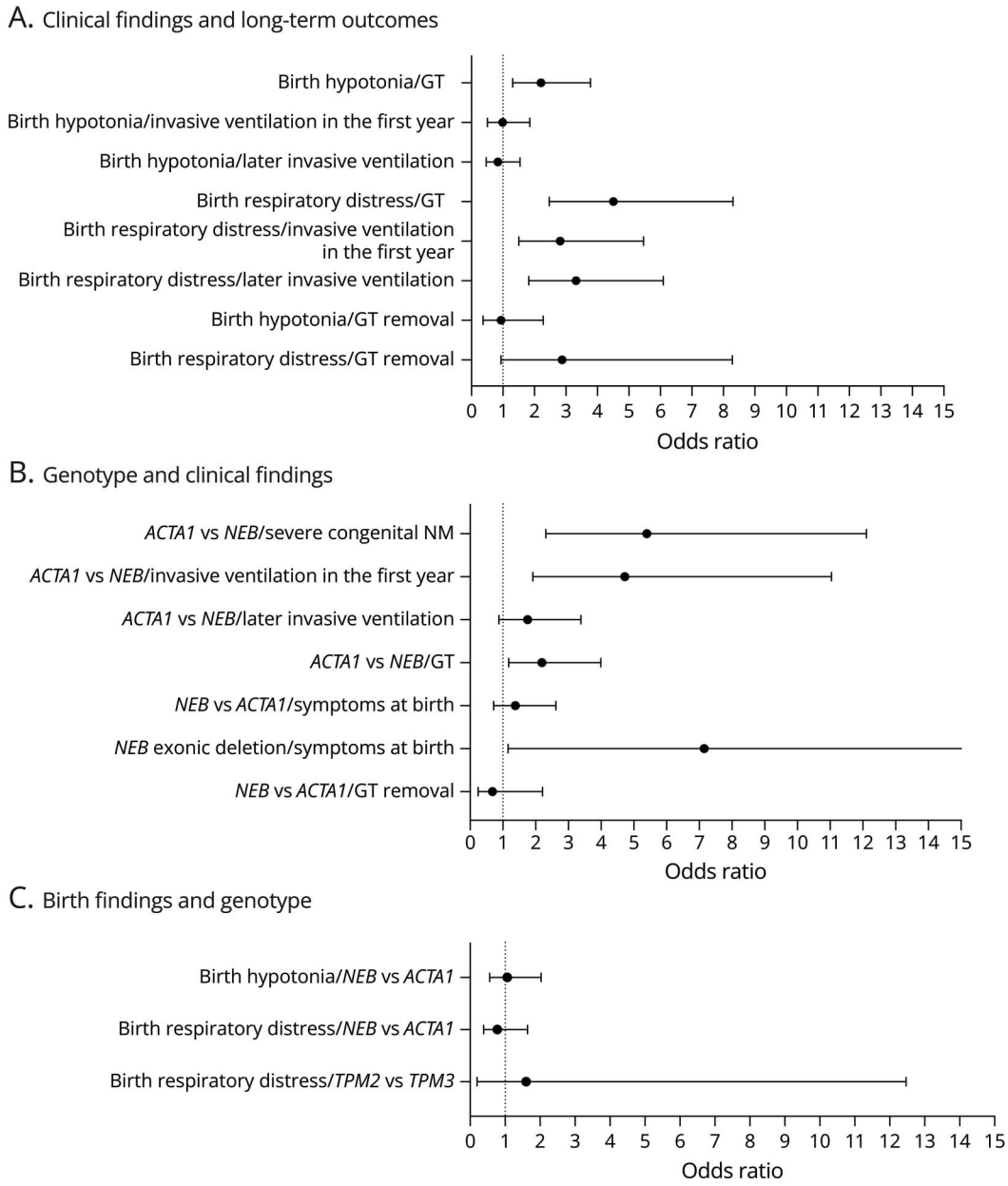


(A) Survival of the overall cohort of patients with available information (dark line, n = 263), compared with survival of the general US population (gray line). Dashed lines represent 96% CI. Of survivors past the age of 60, 1 died of complications related to thyroid cancer and another passed in their sleep because of unknown causes. (B) Survival by genotype: no molecular cause identified (solid dark line, n = 50), *NEB* (dashed dark line n = 95), *ACTA1* (solid gray line, n = 76), and *TPM3* (dashed gray line, n = 18), among patients with available information. Of 15 patients with *TPM3*-NM still alive at last known follow-up, age at last follow-up ranged from 1 year to 36 years with an average of 15.6 years. Pairwise comparisons (using Benjamini-Hochberg correction to adjust for multiple testing) showed that the group without molecular diagnosis (18 of total 50 patients died) and those with *NEB*-NM (13 of 95 patients died) showed a difference in survival, with the *NEB*-NM group having higher rates of survival ($p = 0.0076$). (C) Survival by degree of respiratory assistance before 1 year of age: no respiratory assistance (solid dark line, n = 134), invasive respiratory assistance (dashed line, n = 51), and continuous positive airway pressure or bilevel positive airway pressure (dashed/dotted line, n = 11), among patients with available information. This analysis was based on 196 patients (5 excluded because of missing last follow-up/death time). (D) Survival by finding of respiratory failure at birth: no respiratory failure (dark line, n = 209) and presence of respiratory failure (gray line, n = 54), among patients with available information. Two hundred sixty-three patients were included in analyses (5 with missing information and 4 terminated or stillborn patients were excluded). (E) Time to complete or partial loss of ambulation of the cohort, in patients with available information (n = 212). Additional 53 patients had missing age at loss of ambulation or last follow-up and were excluded from analyses. A total of 153 patients were included in the analyses. Dashed lines represent 96% CI. These analyses omit 3 cases of *TNNT1*-NM.

Clinical Severity Scores

Amburgey et al.[25] calculated severity scores according to the following formula: (6 × ambulatory [no = 1, yes = 0]) + (2 × respiratory support [invasive = 3, noninvasive day and night = 2, noninvasive night only = 1, no support = 0]) + (feeding tube [yes = 1, no = 0]) = severity score. To assess utility, Amburgey scores in our cohort were compared within the following analysis groups: diagnosis category (clinical/histologic, clinical/histologic and molecular, molecular only), genotype, and 2000 NM Classification category for individuals with enough available information to calculate a score. Because data distributions were non-Gaussian, Kruskal-Wallis analysis with Dunn correction for multiple comparisons was used to compare medians. The median score of severe NM (n = 13, median = 13) was not statistically different from that of intermediate NM (n = 41, median = 9), but there were differences in median scores between severe and typical (n = 104, median = 0), severe and mild/juvenile (n = 24, median = 0), and severe and adult onset (n = 11, median = 0), as well as intermediate and typical/congenital

Figure 3 Odds Ratio Analyses



Odds ratios, modeled using the Fisher exact test, for relationships between clinical findings and long-term outcomes (A), genotype and clinical findings (B), and birth findings and genotype (C). Outcomes for each reported association are indicated on Y axes. Dots represent odds ratio, and lines represent 95% CI. Because multiple comparisons were conducted, the expected number of significant results by chance was compared with the number observed. With 18 comparisons and alpha set at 0.05, 0.9 comparisons would be expected to reject the null hypothesis (no difference in odds) by chance. The analysis found 7 comparisons whose 95% CIs exceeded the odds ratio of one, rejecting the null hypothesis. GT = gastrostomy tube.

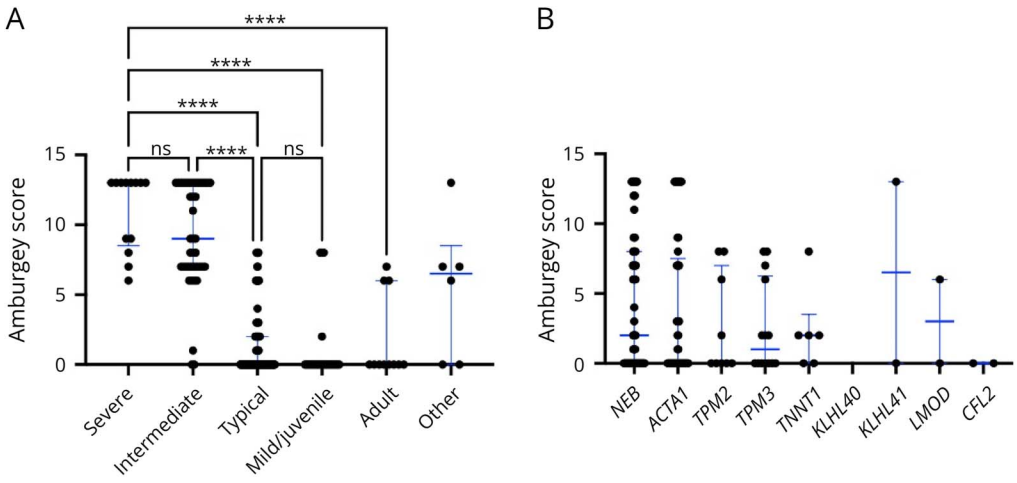
(Figure 4A). There was no difference in median scores of typical and mild/juvenile NM. There were no differences in median scores when comparing Amburgey scores by genotype (Figure 4B).

The 2000 NM Classification Scheme[3] was compared with the updated Sewry NM Classification Scheme[23] using Spearman correlation ($p < 0.0001$) (Figure 5). Using the Fisher exact test, hypotonia at birth and the original severity classes were found not to be independent ($p < 0.001$, OR 0.34, 95% CI 0.29–0.68) and respiratory distress at birth was also not independent of the severity class ($p < 0.001$, OR 0.29, 95% CI 0.17–0.48). Clinical birth findings appeared to correlate with the 2000 NM Classification Scheme as expected (Figure 6).

Descriptive Results

Detailed clinical and neurologic findings, histology, and information on adult-onset cases can be found in eSupplement 1 and eTables 6–11, respectively.

Figure 4 Clinical Severity Scores Broken Down by Clinical Category and Genotype



The Amburgey scoring system related to the 2000 NM Classification Scheme (A) and genotype (B) (*NEB*-NM n = 80, median = 1.5, mean = 4.65; *ACTA1*-NM n = 53, median = 0, mean = 2.76; *TPM2*-NM n = 9, median = 0, mean = 3; *TPM3*-NM n = 14, median = 1, mean = 3.79; *TNNT1*-NM n = 3, median = 0, mean = 6.33; *KLHL40*-NM n = 0; *KLHL41*-NM n = 2, median = 6.5, mean = 3.5; *LMOD3*-NM n = 2, median = 3, mean = 3; *CFL2*-NM n = 2, median = 0, mean = 0). *$p = 0.048$, ****$p < 0.0001$, ns = not significant. The blue vertical lines represent the interquartile range, and the horizontal line represents the median. NM = nemaline myopathy.

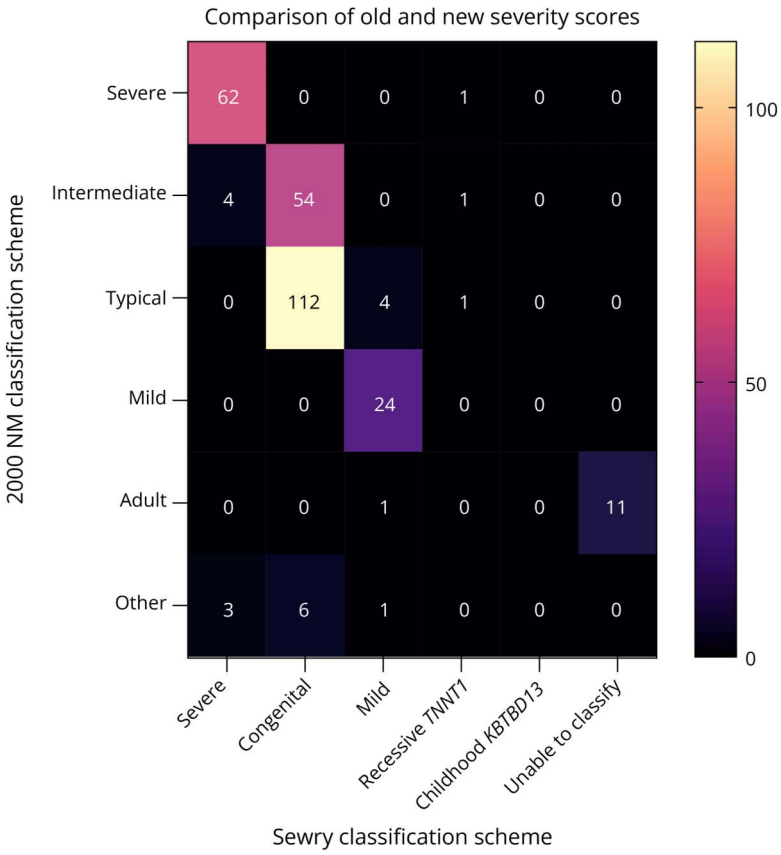
Discussion

Clinical severity classes are helpful to describe the degree of system involvement in individuals with NM but often have to be applied retroactively once certain milestones are achieved or missed. An ideal severity score would be predictive, but currently, there is limited information on early predictors of long-term outcome in NM. As rapid genetic diagnosis is becoming increasingly common in the prenatal and neonatal period, providing accurate prognostic information for families is important. Swift molecular diagnosis of critically ill neonates demonstrates consistent high yield,[59,60] and parents especially value the prognostic information that early and rapid molecular diagnosis can provide.[61,62] In this study, we detail clinical findings and outcomes in a large cohort of patients with NM to identify the range of clinical outcomes associated with specific genotypes and early clinical presentations.

The most common presenting symptom in this cohort of 275 patients was hypotonia (n = 87, 32%). Frequent complications included need for respiratory support and feeding support. Seventy-three patients (27%) required invasive ventilation, and an additional 40 (15%) required some degree of positive pressure ventilation while 105 patients (38%) required a GT at some point in their lives. Sixty individuals (22%) were not ambulatory at the time of data collection (at an age where ambulation would be expected), and 17 (6%) required some assistance with ambulation. Of 131 ambulatory patients, 13 required invasive ventilation and 17 required positive pressure ventilation at some time during recorded follow-up. Of the 13 patients requiring invasive ventilation, 3 were able to discontinue invasive ventilation in childhood (eSupplement 1).

The Amburgey composite scoring system is useful in prospectively following patients to determine severity over time. However, while the scoring system was able to differentiate severe from typical, mild, or adult-onset disease and intermediate from typical disease according to the 2000 NM Classification Scheme, it was not able to clearly differentiate between severe and intermediate NM or typical and mild NM in this cohort. It is important to have early clinical predictors of long-term outcomes, but to be scored, a child must be of walking age, so predicting long-term outcomes is not possible during the first years of life. Our analysis identified several genetic and early clinical risk factors that increase the odds of eventually requiring feeding or respiratory support. Patients with *ACTA1*-NM were more likely to require invasive ventilation in the first year of life than patients with *NEB*-NM. Early clinical predictors of need for invasive ventilation included respiratory distress at birth, and early clinical predictors of requiring a GT included respiratory distress and hypotonia at birth. However, once GT was placed, neither early clinical signs nor *NEB* vs *ACTA1* variant status predicted whether GT could later be removed, perhaps in part because of variable clinical practice as some clinicians and families may opt to leave a GT in place for use in times of illness. A previous study showed equal rates of respiratory and feeding support among 8 individuals with *NEB*-NM and 17 with *ACTA1*-NM,[63] but another study reported 67% feeding tube use among 18 patients with *ACTA1*-NM and 32% among 19 with *NEB*-NM, and need for invasive ventilation in 39% and 25%, respectively, of these patients with *ACTA1*-NM and *NEB*-NM.[25] Similar to this latter study, we show that rates for invasive ventilation (any time in life) were 34% in *ACTA1*-NM and 23% in *NEB*-NM and patients with *ACTA1*-NM were more likely to require invasive ventilation in the first year

Figure 5 Concordance Analysis of Alternate NM Classification Schemes



Comparison of the 2000 NM Classification Scheme[20] and the updated Sewry NM Classification Scheme proposed in 2019.[23] The 2 schemes are significantly correlated (p 0.0001). Color legend and numbers in each box indicate the number of overlapping cases for each severity class. NM = nemaline myopathy.

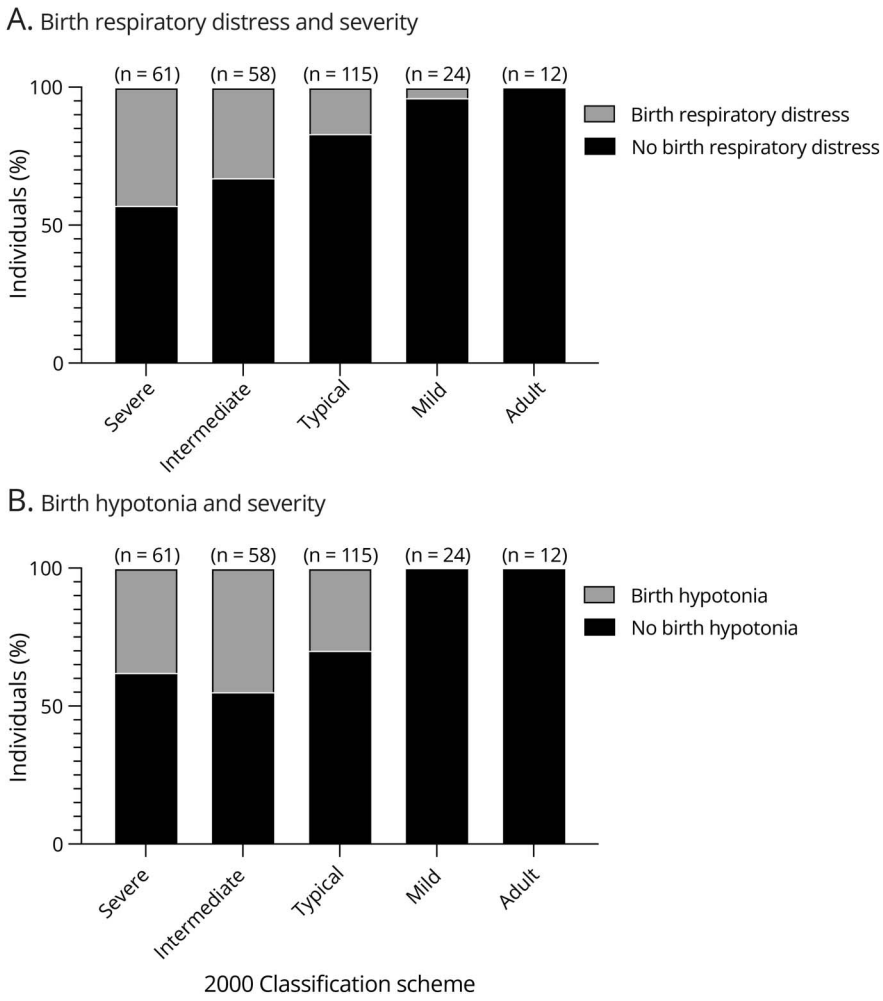
of life compared with patients with *NEB*-NM. The rate of feeding tube use was 57% in *ACTA1*-NM and 37% in *NEB*-NM. Our study also confirms that individuals with *ACTA1*-NM are more likely to have severe disease than individuals with *NEB*-NM (OR 5.4). The impact of the Ashkenazi founder *NEB* exonic deletion (ΔExon55) was evaluated and revealed that patients with *NEB*-NM who were symptomatic at birth were more likely to have the ΔExon55 than other variants in *NEB*, as suggested previously.[27,46] Death was reported more frequently in children with *NEB*-NM because of deletion of exon 55 (3/13 or 23%) than in those with *NEB*-NM because of other pathogenic variants (10/83 or 12%), but this did not meet statistical significance (OR 2.2, 95% CI 0.56–8.99). Prospective studies analyzing both genotype and early clinical predictors will need to be conducted to determine the power of these findings.

Most patients in this cohort were symptomatic in childhood (median 0.13 years, mean 2.8 years), with diagnosis often occurring several years later (median 5.2, mean 8.2 years). Possible contributors to the overall delay in diagnosis may include limitations in genetic testing for patients born in the 20th century and early 2000s and delay in obtaining muscle biopsies, requiring sedation, in less severely affected children. Now that definitive molecular diagnosis is possible earlier, muscle biopsies are often deferred. In this cohort, most patients in each severity class had rods on muscle biopsy and it did not appear that there were major differences in histology among the severity classes (eTable 10).

The median survival of this cohort was 66 years. Survival appeared bimodal with noticeable dropoffs in the first year of life, as well as after age 60 years. Although survival for *NEB*-NM and *ACTA1*-NM groups was not statistically different, it does appear that death occurs earlier in the *ACTA1*-NM group. Decreased survival in patients without a molecular diagnosis compared with patients with *NEB*-NM may reflect that the former group was part of an older cohort when molecular testing was less available and life-prolonging medical interventions were less successful. Predictably, survival in patients with respiratory distress at birth or need for invasive ventilation in the first year of life was lower than in patients without these findings.

A previous study including 91 patients overlapping with the present cohort, but published in 2001 when molecular testing was largely limited to some *ACTA1*-NM and *TPM3*-NM cases, revealed that cardiac disease outside of neonatal critical illness is rare.[54] In our cohort, cardiomyopathy was reported in 12 of 226 informative cases (2 with *NEB*-NM, 5 with

Figure 6 Clinical Presentations at Birth Among Infants Eventually Categorized According to the 2000 NM Classification Scheme



The relationship between birth respiratory distress (A) and birth hypotonia (B) with the 2000 NM Classification Scheme. NM = nemaline myopathy.

ACTA1-NM, 1 with *TPM2*-NM, 4 without molecular diagnosis). That same 2001 study[54] indicated that while respiratory failure in the newborn period was predictive of early mortality, hypotonia at birth was not. This is supported by findings in our extended cohort that neonatal respiratory distress increases odds of need for invasive ventilation and results in concomitant increase in mortality, but neonatal hypotonia did not. In the 2001 cohort, creatine kinase (CK) was infrequently elevated and EMG showed abnormalities in approximately two-thirds of tested patients. Similarly, our study showed elevated CK in only 16 of 116 informative cases and abnormal EMG in 78 of 132 (approximately two-thirds) of informative cases.

The analysis of this cohort faced several limitations because of the retrospective nature of the study. Patient data were collected over a long period and from many different institutions leading to a diversity in treatment approaches, variable recording of clinical assessments (interexaminer variability), challenges with data coding, and missing data. Clinical outcomes were grouped into broad categories to address the variability of clinical terminology in medical records from different institutions. The standard workup, including availability of molecular genetic testing, and treatment evolved over the period of this study and may have affected the characterization of patients and skewed certain outcome measurements. Survival analyses were also likely affected by the long enrollment period as the degree and success of medical management have increased. Not all patients were followed into adulthood, affecting ascertainment of long-term outcomes. Patients entered the study via physician or self-referral, which may have led to a selection bias for more severe cases. Clinical data were collected using a standardized document completed by a physician or family member, which may have introduced recall bias and missing data given the length between events and the time of reporting. Another limitation is the differing diagnostic basis for inclusion because some patients were molecularly defined while others

were included on the basis of clinicopathologic data alone. While this may make it difficult to compare groups for certain analyses, it does reflect the real diagnostic process of NM, which should be considered when evaluating potential long-term predictors of clinical outcomes.

Despite the relatively large size of this NM cohort the significant genotypic variability makes it difficult to ascertain genotype-phenotype relationships because of the many smaller and underpowered subgroup comparisons that would be required. A larger international registry with prospective longitudinal data collection will be necessary to further define genotype-phenotype relationships and could aid in clinical trial recruitment. This study did not assess perceptions of carrying a diagnosis of NM or having a child with NM. It will be important to investigate perceived quality of life among the different severity classes and to involve families with NM in determining meaningful clinical outcomes for potential therapies. As therapies for NM become available, accurate early assessment of severity and prognosis will be crucial to determine eligibility. It will be important to ensure equitable access to care for these therapies. Determination of barriers to access of care and new therapies among individuals with NM should be investigated as new therapies are developed.

As development of therapies for NM progresses, it is important to understand the clinical history of the disease and determine findings detectable early in life that can predict prognosis. This retrospective cohort analysis revealed several features present on examination at birth, such as respiratory distress and hypotonia, which are related to long-term clinical outcomes. Data suggest that patients with *ACTA1*-NM are more likely to have severe congenital disease than patients with *NEB*-NM. This may prioritize therapy development for this group, as these individuals would be most likely to derive measurable benefit from novel therapies. We also show that the Amburgey scoring system can differentiate between clinically distinct severity classes. Because this score cannot be applied until after expected ambulation, early clinical predictors such as those identified in this cohort may help guide selection of candidates for new therapies. The creation of an NM registry would facilitate recruitment for a large prospective longitudinal natural history study, an important next step to inform the design of upcoming clinical trials.

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Author Contributions

C. Hildebrandt: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. C.A. Genetti: major role in the acquisition of data. T. Logvinenko: analysis or interpretation of data. W. Win: major role in the acquisition of data. P. Barraza-Flores: major role in the acquisition of data. L.H. Hayes: drafting/revision of the manuscript for content, including medical writing for content. S. Rockowitz: major role in the acquisition of data. V.-L. Lehtokari: major role in the acquisition of data. S.T. Iannaccone: major role in the acquisition of data. B.T. Darras: major role in the acquisition of data. H. Topaloglu: major role in the acquisition of data. C. Wallgren-Pettersson: major role in the acquisition of data. A.H. Beggs: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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Disclosure

B.T. Darras has served as an ad hoc scientific advisory board member for Audentes Therapeutics, AveXis/Novartis Gene Therapies, Biogen, Pfizer, Sarepta, Vertex and Roche/Genentech; was a Steering Committee Chair for Roche FIREFISH and

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A retrospective cohort analysis of clinical, molecular and histopathological characteristics of 275 patients with Nemaline Myopathy

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A data dictionary specifying the designations and coding of qualitative data analyzed in the study is available separately as file “NM_Data_Dictionary.xlsx”.

eAppendix 1: Additional clinical findings

Incidence of additional clinical findings not directly discussed in manuscript, for patient with available information:

Prenatal History:
For patients where prenatal information was known (n=236), the most common prenatal complication was decreased fetal movements (n=69).

Other common findings included:
Polyhydramnios (34)
Breech presentation (27)
Increased NT (5)
Fetal distress leading to emergent CS (5)
Gestational DM (6)
Bleeding during pregnancy (3)
Placental abruption (3)
Prenatal detection of clubfoot (3)
IUGR (3)
Antepartum hemorrhage (3)
"Abnormal serum screen" (2)
Forceps delivery (2)
Pre-eclampsia (2)
PPROM (1)
Oligohydramnios (1)
HELLP syndrome (1)
Post-partum hemorrhage (1)
Elevated AFP (1)
SGA (1)
Transverse lie (1)
Absence of stomach bubble (1)
Single umbilical artery (1)
Dysplastic pelvic kidney (1)
Echogenic bowel (1)
Choroid plexus cyst (1)
Cystic degeneration of umbilical cord (1)
Clonus in utero (1)
Jittery fetal movements (1)
Shoulder dystocia (1)
IUFD of twin (1)

Additionally, 3 twin pregnancies were delivered.

Birth History:
Gestational age at delivery was known for 230 patients and 191 of these were born at term. The most common abnormal finding at birth in patients where birth exam information was available (n=213) was hypotonia (n=83).

Other common problems included:
Respiratory distress (66)

Apnea (21)
Absence of antigravity movements (32)
Cardiogenic shock (1)

Exam findings at birth included:
Weak cry (7)
Findings consistent with prematurity (5)
Micrognathia (2)
High arched palate (2)
SGA (2)
Ophthalmoplegia (2)
Kyphosis (1)
Scoliosis (1)
Macrocephaly (1)
Bifid thumb (1)
Crumpled ears (1)
Dislocated knee (1)
Tracheoesophageal fistula (1)
Increased startle (1)
Ptosis (1)

Death occurred at delivery or in the first day of life in 6 individuals.

Perinatal complications:
The most common perinatal complication in cases where information was available (n=241) was feeding issues (n=101).

Other complications included:
Respiratory failure (56)
Hypotonia (24)
Continued respiratory distress (14)
Aspiration (7)
Complications attributed to prematurity (7) such as feeding immaturity or bradycardia
Jaundice (4)
Cardiac arrest (2)
Pneumonia (2)
Sepsis (1)
RSV infection (1)
Polycythemia (1)
Cardiogenic shock (1)
Reflux (1)
SVT (1)
Hypoglycemia (1)
Hypoxia of unknown etiology (1)
Cyanosis (1)
Chylothorax (1)
Stridor (1)
Spastic tetraparesis (1)
Brachial plexus birth injury (1)

Presenting Symptoms:

The most common presenting symptoms in individuals where information was available (n=257) were hypotonia (n=87), feeding issues (n=76), respiratory distress (n=69), weakness (n=69), and motor delays (n=54).

Other presenting symptoms included:
Aspiration (14)
Failure to thrive (13)
Contractures (11)
Abnormal gait (11)
Frequent falls (9)
Recurrent respiratory infections (9)
Cardiomyopathy (4)
Hypomimia (2)
Myalgias (2)
Scoliosis (1)
Hyper nasal speech (1)
Back pain (1)
Tongue fasciculations (1)
Foot drop (1)
Tremors (1)
Dysarthria (1)
Arm pain (1)
Clumsiness (1)
Dysphagia (1)
Birth of more severely affected child (1)

eTable 1.

2000 NM Classification Scheme[a]	
Severity Score	Description
Severe	A.1. Severe congenital nemaline myopathy Inclusion criteria: No spontaneous movements at birth; No spontaneous respiration at birth; Contractures at birth; Fractures at birth. Exclusion criteria: Associated features: Cardiomyopathy; Ophthalmoplegia; Unusual distribution of weakness; Intranuclear nemaline bodies.
Intermediate	A.2. Intermediate congenital nemaline myopathy Inclusion criteria: Infantile onset. Breathing and moving at birth, but later in early childhood unable to achieve respiratory independence or; unable to achieve sitting or; unable to achieve walking. Contractures developing in early childhood. Use of wheelchair before the age of 11 years
Typical	A.3. The typical form of congenital nemaline myopathy Inclusion criteria: Onset in early childhood. Weakness especially pronounced in the facial, bulbar and respiratory muscles, and in the neck flexors. Proximal > distal weakness initially. Milestones delayed but reached. Later distal involvement. Slowly progressive or non-progressive course. Exclusion criteria: No spontaneous movements at birth. No spontaneous respiration at birth. Contractures at birth. Fractures at birth. Unable to be establish independent respiration. Unable to learn to sit or walk. Use of wheelchair before the age of 11 years. Adult or late childhood onset.
Mild	A.4. Mild childhood or juvenile onset nemaline myopathy Childhood or juvenile onset. No facial weakness. No foot drop
Adult	A.5. Adult-onset forms of nemaline myopathy Inclusion criterion: Adult onset.
Other	A.6. Other forms of nemaline myopathy Inclusion criteria: Cardiomyopathy; Ophthalmoplegia; Unusual distribution of weakness; Intranuclear nemaline bodies.

eTable 1. (Continued)

Sewry Classification Scheme[b]
Severe nemaline myopathy (with contractures or fractures at birth, or with no respiratory efort or no movements at birth) (ACTA1, NEB, LMOD-3, KLHL40, KLHL41, TNNT3, TPM2, TPM3)
Congenital nemaline myopathy (with perinatal onset and milestones delayed but reached) (NEB, ACTA1, CFL-2, TPM2)
Mild (childhood or juvenile onset) nemaline myopathy (ACTA1, NEB, TPM2, TPM3, KBTBD13, MYPN, dominant mutations inTNNT1)
Recessive TNNT1 (Amish) nemaline myopathy
Childhood-onset nemaline myopathy with slowness of movements and core-rod histology (KBTBD13)

[a] 2000 NM Classification scheme outlined in Wallgren-Pettersson C, Laing NG. Report of the 70th ENMC International Workshop: nemaline myopathy, 11-13 June 1999, Naarden, The Netherlands. *Neuromuscul Disord*. 2000;10(4-5):299-306.

[b] 2019 revised classification scheme as per Sewry CA, Laitila JM, Wallgren-Pettersson C. Nemaline myopathies: a current view. *J Muscle Res Cell Motil*. 2019;40(2):111-126. doi:10.1007/s10974-019-09519-9.

eTable 2. Patients with *ACTA1* variants.

ID	Dx[a]	Severity[b]	Age (status)[c]	Genotype (NM_001100.4)	Predicted proteins (NP_001091.1)	Genomic Variants (NC_000001.11)
8-2	NM[1-5]	Intermediate	0.45 (d)	c.[846C>G];[846=]	p.[(Asn282Lys)];[(Asn282=)]	g.[229431865G>C];[229431865=]
18-3	NM[1-5]	Typical	1.3 (a)	c.[551G>A];[551=]	p.[(Gly184Asp)];[(Gly184=)]	g.[229432335C>T];[229432335=]
25-1	NM[1-5]	Severe	0.02 (d)	c.[863A>G];[863=]	p.[(Asp288Gly)];[(Asp288=)]	g.[229431848T>C];[229431848=]
35-1	NM[2-5]	Intermediate	0.4 (d)	c.[553C>G];[553=]	p.[(Arg185Gly)];[(Arg185=)]	g.[229432333G>C];[229432333=]
80-1	NM[2, 4, 5]	Typical	70 (a)	c.[128A>G];[128=]	p.[(Gln43Arg)];[(Gln43=)]	g.[229432988T>C];[229432988=]
80-2	NM[2, 4, 5]	Typical	25 (a)	c.[128A>G];[128=]	p.[(Gln43Arg)];[(Gln43=)]	g.[229432988T>C];[229432988=]
81-1	NM[6]	Severe	18 (a)	c.[358A>G];[358=]	p.[(Lys120Glu)];[(Lys120=)]	g.[229432652T>C];[229432652=]
86-1	NM[1-5]	Intermediate/IRM	0.1 (d)	c.[124C>T];[124=]	p.[(His42Tyr)];[(His42=)]	g.[229432992G>A];[229432992=]
90-1	NM[2-5]	Typical	33 (a)	c.[1123A>C];[1123=]	p.[(Lys375Gln)];[(Lys375=)]	g.[229431510T>G];[229431510=]
90-2	NM[2-5]	Typical	5 (a)	c.[1123A>C];[1123=]	p.[(Lys375Gln)];[(Lys375=)]	g.[229431510T>G];[229431510=]
95-1	NM[2, 4, 5, 7, 8]	Typical	7 (a)	c.[235A>G];[235=]	p.[(Thr79Ala)];[(Thr79=)]	g.[229432775T>C];[229432775=]
103-1	NM[2, 4, 5]	Severe	0 (d)	c.[224A>T];[224=]	p.[(His75Leu)];[(His75=)]	g.[229432786T>A];[229432786=]
104-1	NM[2, 4, 5]	Mild	6 (a)	c.[743A>G];[743=]	p.[(Gln248Arg)];[(Gln248=)]	g.[229432059T>C];[229432059=]
104-2	NM[2, 4, 5]	Typical	40 (a)	c.[743A>G];[743=]	p.[(Gln248Arg)];[(Gln248=)]	g.[229432059T>C];[229432059=]
104-3	NM[2, 4, 5]	Typical	40 (a)	c.[743A>G];[743=]	p.[(Gln248Arg)];[(Gln248=)]	g.[229432059T>C];[229432059=]
104-4	NM[2, 4, 5]	Typical	2 (a)	c.[743A>G];[743=]	p.[(Gln248Arg)];[(Gln248=)]	g.[229432059T>C];[229432059=]
104-5	NM[2, 4, 5]	Mild	unknown	c.[743A>G];[743=]	p.[(Gln248Arg)];[(Gln248=)]	g.[229432059T>C];[229432059=]
108-1	NM[2-5, 9]	Typical	11 (a)	c.[407T>C];[407=]	p.[(Val136Ala)];[(Val136=)]	g.[229432603A>G];[229432603=]
115-1	NM[2-5]	Severe	1.5 (a)	c.[109G>C];[109=]	p.[(Val37Leu)];[(Val37=)]	g.[229433007C>G];[229433007=]
116-1	NM	Intermediate	8 (a)	c.[542A>G];[542=]	p.[(Asp181Gly)];[(Asp181=)]	g.[229432344T>C];[229432344=]
117-1	NM[2, 4, 5]	Severe	0.16 (d)	c.[436del];[782A>T]	p.[(Ala146ProfsTer46)];[(Glu261Val)]	g.[229432574del];[229432020T>A]
120-1	NM[2, 4, 5]	Typical	50 (d)	c.[197T>A];[197=]	p.[(Ile66Asn)];[(Ile66=)]	g.[229432813A>T];[229432813=]
120-3	NM[2, 4, 5]	Typical	9 (a)	c.[197T>A];[197=]	p.[(Ile66Asn)];[(Ile66=)]	g.[229432813A>T];[229432813=]
173-1	NM	Severe	14 (a)	c.[541del];[514del]	p.[(Asp181ThrfsTer11)]; [(Ala172ArgfsTer20)]	g.[229432346del];[229432372del]
180-1	NM[4, 6]	Typical	0.4 (a)	c.[551G>A];[551=]	p.[(Gly184Asp)];[(Gly184=)]	g.[229432335C>T];[229432335=]
181-1	NM[2-5]	Intermediate	13 (a)	c.[808G>T];[808=]	p.[(Gly270Cys)];[(Gly270=)]	g.[229431994C>A];[229431994=]
188-1	NM[2, 5]	Adult	52 (d)	c.[1031del];[1031=]	p.[(Gly344AlafsTer77)];[(Gly344=)]	g.[229431603del];[229431602=]
199-1	NM[2, 4, 5]	Mild	21 (a)	c.[687G>C];[687=]	p.[(Met229Ile)];[(Met229=)]	g.[229432115C>G];[229432115=]
220-1	NM[2, 4, 5]	Severe	7 (a)	c.[229A>C];[229=]	p.[(Ile77Leu)];[(Ile77=)]	g.[229432781T>G];[229432781=]

eTable 2. Patients with *ACTA1* variants (Cont'd).

ID	Dx[a]	Severity[b]	Age (status)[c]	Genotype (NM_001100.4)	Predicted proteins (NP_001091.1)	Genomic Variants (NC_000001.11)
226-1	NM[2, 4, 5]	Severe	0.1 (d)	c.[443G>A];[443=]	p.[(Gly148Asp)];[(Gly148=)]	g.[229432567C>T];[229432567=]
232-1	NM[5]	Severe/IRM	0.08 (d)	c.[487C>G];[487=]	p.[(His163Asp)];[(His163=)]	g.[229432399G>C];[229432399=]
234-1	NM[2, 4, 5]	Severe	8.6 (d)	c.[758G>A];[758=]	p.[(Gly253Asp)];[(Gly253=)]	g.[229432044C>T];[229432044=]
256-1	NM	Typical/IRM	16 (a)	c.[455G>C];[455=]	p.[(Gly152Ala)];[(Gly152=)]	g.[229432431C>G];[229432431=]
307-1	NM[2, 4, 5]	Severe	6.5 (a)	c.[224A>G];[224=]	p.[(His75Arg)];[(His75=)]	g.[229432786T>C];[229432786=]
308-1	NM[2, 4, 5]	Severe	0.08 (d)	c.[119C>T];[119=]	p.[(Pro40Leu)];[(Pro40=)]	g.[229432997G>A];[229432997=]
349-1	NM[2, 5, 7, 8]	Typical	21 (a)	c.[253G>A];[253=]	p.[(Glu85Lys)];[(Glu85=)]	g.[229432757C>T];[229432757=]
349-2	NM[2, 5]	Typical	21 (a)	c.[353G>A];[353=]	p.[(Arg118His)];[(Arg118=)]	g.[229432657C>T];[229432654=]
350-1	NM[2, 5]	Severe	0.25 (d)	c.[595G>A];[595=]	p.[(Gly199Ser)];[(Gly199=)]	g.[229432291C>T];[229432291=]
503-1	NM	Severe	0.002 (d)	c.[236C>T];[236=]	p.[(Thr79Ile)];[(Thr79=)]	g.[229432774G>A];[229432774=]
671-1	NM[10]	Intermediate	1.5 (a)	c.[109G>C];[109=]	p.[(Val37Leu)];[(Val37=)]	g.[229433007C>G];[229433007=]
758-1	NM	Severe	0.13 (d)	c.[413T>C];[413=]	p.[(Ile138Thr)];[(Ile138=)]	g.[229432597A>G];[229432597=]
760-1	NM	Mild	18 (a)	c.[743A>G];[743=]	p.[(Gln248Arg)];[(Gln248=)]	g.[229432059T>C];[229432059=]
845-1	NM	Intermediate	7 (a)	c.[478G>C];[478=]	p.[(Gly160Arg)];[(Gly160=)]	g.[229432408C>G];[229432408=]
922-1	NM[7, 8, 10]	Typical	0.75 (a)	c.[169G>C];[169=]	p.[(Gly57Arg)];[(Gly57=)]	g.[229432841C>G];[229432841=]
927-1	NM[7, 8]	Typical	6 (a)	c.[773G>A];[773=]	p.[(Arg258His)];[(Arg258=)]	g.[229432029C>T];[229432029=]
935-1	NM	Severe	0.5 (a)	c.[800C>G];[800=]	p.[(Ser267Cys)];[(Ser267=)]	g.[229432002G>C];[229432002=]
945-1	NM[10]	Severe	2.8 (d)	c.[109G>T];[109=]	p.[(Val37Leu)];[(Val37=)]	g.[229433007C>A];[229433007=]
953-1	NM[10]	Intermediate	1 (a)	c.[758G>A];[758=]	p.[(Gly253Asp)];[(Gly253=)]	g.[229432044C>T];[229432044=]
999-1	NM	Intermediate	27 (a)	c.[109G>T];[109=]	p.[(Val37Leu)];[(Val37=)]	g.[229433007C>A];[229433007=]
1004-1	NM	Intermediate	3 (d)	c.[814G>C];[814=]	p.[(Glu272Gln)];[(Glu272=)]	g.[229431897C>G];[229431897=]
1010-1	CFTD	Severe	0.25 (d)	c.[290G>C];[290=]	p.[(Arg97Pro)];[(Arg97=)]	g.[229432720C>G];[229432720=]
1010-2	CFTD	Typical	30 (a)	c.[290G>C];[290=]	p.[(Arg97Pro)];[(Arg97=)]	g.[229432720C>G];[229432720=]
1010-4	CFTD	Typical	61 (a)	c.[290G>C];[290=]	p.[(Arg97Pro)];[(Arg97=)]	g.[229432720C>G];[229432720=]
1016-1	NM[10]	Typical	12 (a)	c.[553C>G];[553=]	p.[(Arg185Gly)];[(Arg185=)]	g.[229432333G>C];[229432333=]
1029-1	NM	Intermediate/IRM	2 (a)	c.[627G>C];[627=]	p.[(Glu209Asp)];[(Glu209=)]	g.[229432175C>G];[229432175=]
1034-1	NM[10]	Typical	13 (a)	c.[541G>A];[541=]	p.[(Asp181Asn)];[(Asp181=)]	g.[229432345C>T];[229432345=]
1060-1	NM	Severe	1 (a)	c.[808G>T];[808=]	p.[(Gly270Cys)];[(Gly270=)]	g.[229431994C>A];[229431994=]
1087-1	CFTD	Typical	38 (a)	c.[142G>A];[142=]	p.[(Gly48Ser)];[(Gly48=)]	g.[229432868C>T];[229432868=]

eTable 2. Patients with *ACTA1* variants (Cont'd).

ID	Dx[a]	Severity[b]	Age (status)[c]	Genotype (NM_001100.4)	Predicted proteins (NP_001091.1)	Genomic Variants (NC_000001.11)
1091-1	CFTD	Severe	0.135 (d)	c.[442G>A];[442=]	p.[(Gly148Ser)];[(Gly148=)]	g.[229432568C>T];[229432568=]
1104-1	NM	Typical	10 (a)	c.[802T>C];[802=]	p.[(Phe268Leu)];[(Phe268=)]	g.[229432000A>G];[229432000=]
1117-1	CFTD	Severe	10 (a)	c.[79G>A];[79=]	p.[(Asp27Asn)];[(Asp27=)]	g.[229433037C>T];[229433037=]
1150-1	NM	Severe	0.6 (a)	c.[355G>C];[355=]	p.[(Glu119Gln)];[(Glu119=)]	g.[229432655C>G];[229432655=]
1298-1	CFTD	Severe	4 (a)	c.[142G>T];[142=]	p.[(Gly48Cys)];[(Gly48=)]	g.[229432868C>A];[229432868=]
1351-4	NM	Typical	5 (a)	c.[110T>C];[110=]	p.[(Val37Ala)];[(Val37=)]	g.[229433006A>G];[229433006=]
1351-6	NM	Typical	8.75 (a)	c.[110T>C];[110=]	p.[(Val37Ala)];[(Val37=)]	g.[229433006A>G];[229433006=]
1351-7	NM	Typical	0.75 (a)	c.[110T>C];[110=]	p.[(Val37Ala)];[(Val37=)]	g.[229433006A>G];[229433006=]
1369-1	NM	Severe	5.5 (a)	c.[285_286del];[644A>T]	p.[(Glu95AspfsTer32)]; [(Lys215Met)]	g.[229432724_229432725del]; [229432158T>A]
1376-1	NM	Severe	5 (a)	c.[739G>C];[739=]	p.[(Gly247Arg)];[(Gly247=)]	g.[229432063C>G];[229432063=]
1394-1	NM	Typical	12 (a)	c.[925C>T];[925=]	p.[(Pro309Ser)];[(Pro309=)]	g.[229431786G>A];[229431786=]
1411-1	CFTD	Intermediate	2.8 (a)	c.[1000C>T];[1000=]	p.[(Pro334Ser)];[(Pro334=)]	g.[229431633G>A];[229431633=]
1414-1	NM	Intermediate	4 (a)	c.[130-5T>A];[130-5=]	p.[?];[=]	g.[229432885A>T];[229432885=]
1423-1	NM	Severe	48 (a)	c.[1132T>C];[1132=]	p.[(Ter378GlnextTer47)];[(Ter378=)]	g.[229431501A>G];[229431501=]
1424-1	NM[10]	Typical	0.25 (a)	c.[347C>T];[347=]	p.[(Ala116Val)];[(Ala116=)]	g.[229432663G>A];[229432663=]
1458-1	NCM	Intermediate	0.25 (d)	c.[595G>A];[595=]	p.[(Gly199Ser)];[(Gly199=)]	g.[229432291C>T];[229432291=]
1494-1	CM	Typical	2 (a)	c.[39C>A];[39=]	p.[(Asp13Glu)];[(Asp13=)]	g.[229433077G>T];[229433077=]
1494-3	CM	Mild	39 (a)	c.[39C>A];[39=]	p.[(Asp13Glu)];[(Asp13=)]	g.[229433077G>T];[229433077=]
1494-4	CM	Typical	4.8 (a)	c.[39C>A];[39=]	p.[(Asp13Glu)];[(Asp13=)]	g.[229433077G>T];[229433077=]
1495-1	NM	Intermediate	13 (a)	c.[1130T>G];[1130=]	p.[(Phe377Cys)];[(Phe377=)]	g.[229431503A>C];[229431503=]
1495-3	NM	Typical	5 (a)	c.[1130T>G];[1130=]	p.[(Phe377Cys)];[(Phe377=)]	g.[229431503A>C];[229431503=]

[a]Referring clinicopathological diagnosis and previous publications. "NM" = nemaline myopathy, "CFTD" = congenital fiber type disproportion, "NCM" = nonspecific congenital myopathy, "CM" = cardiomyopathy.

[b]Clinical severity according to the 2000 NM classification scheme. "IRM" = Intranuclear Rod Myopathy.

[c]Age in years at last known status. "a" = alive, "d" = deceased.

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9. Sanoudou D, Corbett MA, Han M, et al. Skeletal muscle repair in a mouse model of nemaline myopathy. Hum Mol Genet 2006;15:2603-2612.
10. Amburgey K, Acker M, Saeed S, et al. A Cross-Sectional Study of Nemaline Myopathy. Neurology 2021;96:e1425-e1436.

eTable 3. Patients with variants in the *NEB* gene.

ID	Dx[a]	Severity[b]	Age (Status)[c]	Genotype (NM_001271208.2)	Predicted proteins (NP_001258137.2)	Genomic Variants (NC_000002.12)
1-1	NM	Typical	29 (a)	c.[5152C>T];[?]	p.[(Gln1718Ter)];[?]	g.[151665419G>A];[?]
2-2	NM[1]	Typical	18 (a)	c.[7431+1919_7536+374del];[24770_24771del]	p.[(?)];[Phe8257Ter)]	g.[151645758_151648259del];[151493782_151493783del]
4-4	NM[2-5]	Intermediate	13 (d)	c.[7431+1919_7536+374del];[7431+1919_7536+374del]	p.[(?)];[(?)]	g.[151645758_151648259del];[151645758_151648259del]
4-5	NM[2, 3, 5]	Intermediate	21 (a)	c.[7431+1919_7536+374del];[7431+1919_7536+374del]	p.[(?)];[(?)]	g.[151645758_151648259del];[151645758_151648259del]
6-4	NM[6]	Typical	6.5 (a)	c.[294+2T>C];[2319C>G]	p.[(?)];[Tyr773Ter)]	g.[151727689A>G];[151688388G>C]
6-5	NM[6]	Typical	6 (a)	c.[294+2T>C];[2319C>G]	p.[(?)];[Tyr773Ter)]	g.[151727689A>G];[151688388G>C]
7-3	NM[6]	Typical	14 (a)	c.[928-1G>A];[1657_1665delinsTTTATAAT]	p.[(?)];[Ala553PhefsTer4)]	g.[151709764C>T];[151695587_151695595delinsATTATAAA]
7-6	NM[6, 7]	Typical	8 (a)	c.[928-1G>A];[1657_1665delinsTTTATAAT]	p.[(?)];[Ala553PhefsTer4)]	g.[151709764C>T];[151695587_151695595delinsATTATAAA]
9-2	NM	Typical	15 (a)	c.[3691del];[(17535+1_17536-1)_(23940+1_23941-1)del]	p.[(His1231IlefsTer38)];[(?)]	g.[151677648del];[(151502888_151503348)_(151568717_151569269)del]
11-1	NM	Typical	6 (a)	c.24480_24483del(;)11181+323_11340delinsCACATTGGTGCCCGAGCTATACGT	p.(Asn8160LysfsTer19)(;)(?)	g.151496959_151496962del(;)151614537_151617041delinsACGTATAGCTCGGGCACCAATGTG
12-3	NM[8]	Typical	28 (a)	c.[7964A>G];[11628G>A]	p.[(Tyr2655Cys)];[Trp3876Ter)]	g.[151643346T>C];[151612363C>T]
12-4	NM	Typical	11.5 (a)	c.[7964A>G];[11628G>A]	p.[(Tyr2655Cys)];[Trp3876Ter)]	g.[151643346T>C];[151612363C>T]
16-2	NM[9, 10]	Severe	0.08 (d)	c.[12048_12049del];[1152+1G>T]	p.[(Lys4017ArgfsTer10)];[(?)]	g.[151610091_151610092del];[151706880C>A]
16-4	NM[7, 9, 10]	Severe	0 (d)	c.[12048_12049del];[1152+1G>T]	p.[(Lys4017ArgfsTer10)];[(?)]	g.[151610091_151610092del];[151706880C>A]
19-1	NM	Intermediate	6 (a)	c.[20253del];[24684G>A]	p.[(Val6752SerfsTer4)];[Ser8228=)]	g.[151547643del];[151494161C>T]
23-2	NM[10]	Intermediate	15 (d)	c.[20659C>T];[3255+1G>A]	p.[(Arg6887Ter)];[(?)]	g.[151541470G>A];[151679720C>T]
24-2	NM	Intermediate	18 (a)	c.[2051del];[1493A>G]	p.[(Ser684ThrfsTer11)];[Asp498Gly)]	g.[151692114del];[151696713T>C]
26-2	NM[3, 4, 7, 11-13]	Intermediate	10 (a)	c.[7431+1919_7536+374del];[7431+1919_7536+374del]	p.[(?)];[(?)]	g.[151645758_151648259del];[151645758_151648259del]
27-1	NM	Mild	28 (a)	c.[10059C>A];[22696-1G>A]	p.[(Tyr3353Ter)];[(?)]	g.[151627607G>T];[151519070C>T]
28-2	NM	Intermediate	15.75 (a)	c.[24771del];[17118+1G>A]	p.[(Phe8257LeufsTer10)];[(?)]	g.[151493783del];[151570496C>T]
29-1	NM	Intermediate	17 (a)	c.[24380_24383dup];[?]	p.[(His8128GlnfsTer8)];[?]	g.[151497650_151497653dup];[?]
31-2	NM	Mild	8.5 (a)	c.[9046C>T];[?]	p.[(Arg3016Ter)];[?]	g.[151636283G>A];[?]

eTable 3. Patients with variants in the *NEB* gene (Cont'd).

ID	Dx[a]	Severity[b]	Age (Status)[c]	Genotype (NM_001271208.2)	Predicted proteins (NP_001258137.2)	Genomic Variants (NC_000002.12)
32-2	NM	Mild	27 (a)	c.[24317T>A];[12330+5G>A]	p.[(Leu8106Ter)];[(?)]	g.[151497714A>T];[151609804C>T]
56-1	NM	Typical	2.3 (a)	c.[8516G>A];[?]	p.[(Trp2839Ter)];[?]	g.[151640524C>T];[?]
76-1	NM[5]	Typical	15 (d)	c.[5722del];[?]	p.[(Ser1908AlafsTer8)];[?]	g.[151663589del];[?]
88-1	NM	Typical/Opht	1.5 (a)	c.[6487C>T];[?]	p.[(Gln2163Ter)];[?]	g.[151656161G>A];[?]
88-2	NM	Typical	4.5 (a)	c.[6487C>T];[?]	p.[(Gln2163Ter)];[?]	g.[151656161G>A];[?]
94-1	NM	Intermediate	5.5 (a)	c.[24316_24317dup];[?]	p.[(Leu8106PhefsTer75)];[?]	g.[151497714_151497715dup];[?]
99-1	NM[3]	Intermediate	3.5 (d)	c.[7431+1919_7536+374del];[2212-1G>C]	p.[(?)];[(?)]	g.[151645758_151648259del];[151690826C>G]
130-1	NM	Intermediate	8 (d)	c.[1215dup];[?]	p.[(Lys406GlnfsTer13)];[?]	g.[151697590dup];[?]
139-1	NM	Intermediate	1.6 (a)	c.[24282_24283del];[?]	p.[(Arg8094SerfsTer9)];[?]	g.[151498293_151498294del];[?]
146-1	NM	Typical	9 (a)	c.[19102-10_19102-4del];[20122G>A]	p.[(?)];[Val6708Ile)]	g.[151561112_151561118del];[151548343C>T]
152-1	NM[1]	Typical	16 (a)	c.[24632_24633del];[1470+1G>A]	p.[(Pro8211ArgfsTer4)];[(?)]	g.[151494212_151494213del];[151697147C>T]
153-1	NM[1, 10]	Typical	22 (a)	c.[2943+1G>A];[1152+1G>A]	p.[(?)];[(?)]	g.[151682661C>T];[151706880C>T]
170-1	NM	Typical	27 (a)	c.[410A>G];[?]	p.[(Tyr137Cys)];[?]	g.[151724954T>C];[?]
170-4	NM	Typical	23 (a)	c.[410A>G];[?]	p.[(Tyr137Cys)];[?]	g.[151724954T>C];[?]
174-1	NM[3-5]	Intermediate	5 (a)	c.[7431+1919_7536+374del];[7431+1919_7536+374del]	p.[(?)];[(?)]	g.[151645758_151648259del];[151645758_151648259del]
176-1	NM	Typical	15 (a)	c.[4300-2A>G];[1998G>A]	p.[(?)];[Glu666=)]	g.[151671231T>C];[151692261C>T]
178-1	NM	Typical	45 (a)	c.[24693C>G];[?]	p.[(Tyr8231Ter)];[?]	g.[151493859G>C];[?]
179-1	NM	Mild	60 (a)	c.[24282_24283del];[?]	p.[(Arg8094SerfsTer9)];[?]	g.[151498293_151498294del];[?]
182-1	NM	Intermediate	20 (a)	c.[24500_24503dup];[13704T>A]	p.[(Leu8168PhefsTer30)];[Tyr4568Ter)]	g.[151496364_151496367dup];[151600526A>T]
184-1	NM	Mild	2.5 (a)	c.[937C>T];[?]	p.[(Gln313Ter)];[?]	g.[151709754G>A];[?]
202-1	NM	Typical	46 (a)	c.[1263dup];[?]	p.[(Tyr422IlefsTer2)];[?]	g.[151697457dup];[?]
222-1	NM	Intermediate	4 (a)	c.[7228-1G>A];[927A>G]	p.[(?)];[Thr309=)]	g.[151650380C>T];[151710434T>C]
227-1	NM[8, 10]	Severe	0.1 (d)	c.[7647C>G];[9046C>T]	p.[(Tyr2549Ter)];[Arg3016Ter)]	g.[151644127G>C];[151636283G>A]
235-1	NM[3, 4]	Intermediate	15 (a)	c.[7431+1919_7536+374del];[7431+1919_7536+374del]	p.[(?)];[(?)]	g.[151645758_151648259del];[151645758_151648259del]
235-3	NM[3, 4]	Severe	10 (a)	c.[7431+1919_7536+374del];[7431+1919_7536+374del]	p.[(?)];[(?)]	g.[151645758_151648259del];[151645758_151648259del]

eTable 3. Patients with variants in the *NEB* gene (Cont'd).

ID	Dx[a]	Severity[b]	Age (Status)[c]	Genotype (NM_001271208.2)	Predicted proteins (NP_001258137.2)	Genomic Variants (NC_000002.12)
236-1	NM	Mild	18 (a)	c.[24218C>A];[?]	p.[(Ser8073Ter)];[?]	g.[151499299G>T];[?]
238-1	NM	Typical	6 (a)	c.[3567+1G>A];[?]	p.[(?)];[?]	g.[151677875C>T];[?]
238-2	NM	Typical	10.5 (a)	c.[3567+1G>A];[?]	p.[(?)];[?]	g.[151677875C>T];[?]
255-3	NM[1]	Intermediate	6 (a)	c.[24664_24665del];[24664_24665del]	p.[(Asn8222SerfsTer5)];[Asn8222SerfsTer5)]	g.[151494180_151494181del];[151494180_151494181del]
258-2	NM[11, 12]	Typical	5 (a)	c.[3567+3_3567+7del];[18124C>T]	p.[(?)];[Gln6042Ter)]	g.[151677869_151677873del];[151567200G>A]
312-1	NM	Typical	5 (d)	c.[24771del];[9619-2A>G]	p.[(Phe8257LeufsTer10)];[(?)]	g.[151493783del];[151630821T>C]
353-1	NM	Typical	8 (a)	c.[1172_1173del];[24314_24317dup]	p.[(Tyr391Ter)];[Leu8106PhefsTer30)]	g.[151697629_151697630del];[151497714_151497717dup]
545-1	NM	Mild	17.5 (a)	c.[5971-2A>C];[?]	p.[(?)];[?]	g.[151659171T>G];[?]
545-2	NM	Mild	14 (a)	c.[5971-2A>C];[?]	p.[(?)];[?]	g.[151659171T>G];[?]
709-1	NM	Typical	42 (a)	c.[21210T>G];[2559del]	p.[(Tyr7070Ter)];[Met855Ter)]	g.[151535793A>C];[151687499del]
747-1	NM	Intermediate	5 (a)	c.[24190_24193dup];[8031_8041del]	p.[(Lys8065SerfsTer9)];[Lys2677AsnfsTer7)]	g.[151499325_151499328dup];[151643269_151643279del]
807-1	NM	Typical	19 (a)	c.[18367-1_18370del];[?]	p.[(?)];[?]	g.[151565146_151565150del];[?]
866-1	NM	Severe	3.25 (a)	c.[24502_24503dup];[24502_24503dup]	p.[(Leu8168PhefsTer13)];[Leu8168PhefsTer13)]	g.[151496364_151496365dup];[151496364_151496365dup]
890-1	NM	Adult	51 (a)	c.[24654_24655del];[?]	p.[(Arg8218SerfsTer9)];[?]	g.[151494194_151494195del];[?]
910-1	NM	Typical	49 (a)	c.[12160del];[8381A>T]	p.[(Trp4054GlyfsTer12)];[Tyr2794Phe)]	g.[151609979del];[151640659T>A]
920-1	NM[13]	Typical	2.75 (a)	c.[647del];[(3879+1_3880-1)_(3987+1_3988-1)del]	p.[(Ser216IlefsTer12)];[(?)]	g.[151723452del];[(151672681_151674476)_(151674585_151675286)del]
941-1	NM[13]	Typical	3.5 (a)	c.[24156del];[24156del]	p.[(Ile8053PhefsTer127)];[Ile8053PhefsTer127)]	g.[151499363del];[151499363del]
974-1	NM[11, 12]	Intermediate	0.8 (d)	c.[7431+1916_7536+372del];[24840_24841del]	p.[(?)];[Arg8280SerfsTer2)]	g.[151645759_151648261del];[151493387_151493388del]
976-1	NM	Severe	0.125 (d)	c.[22816A>T];[?]	p.[(Lys7606Ter)];[?]	g.[151518407T>A];[?]
988-1	NM[11-13]	Intermediate	0.4 (a)	c.[1152+1G>A];[17013+1G>T]	p.[(?)];[(?)]	g.[151706880C>T];[151575694C>A]
1027-1	NM[8]	Typical	12 (a)	c.[17581G>A];[1997A>T]	p.[(Asp5861Asn)];[(Glu666Val)-splice]	g.[151568671C>T];[151692262T>A]
1027-2	NM	Typical	9 (a)	c.[17581G>A];[1997A>T]	p.[(Asp5861Asn)];[(Glu666Val)-splice]	g.[151568671C>T];[151692262T>A]

eTable 3. Patients with variants in the *NEB* gene (Cont'd).

ID	Dx[a]	Severity[b]	Age (Status)[c]	Genotype (NM_001271208.2)	Predicted proteins (NP_001258137.2)	Genomic Variants (NC_000002.12)
1028-1	NM[13]	Intermediate	19.7 (a)	c.[10899G>A];[19944G>A]	p.[(Trp3633Ter)];[Ser6648=)]	g.[151618452C>T];[151551738C>T]
1028-4	NM	Intermediate	3 (d)	c.[10899G>A];[19944G>A]	p.[(Trp3633Ter)];[Ser6648=)]	g.[151618452C>T];[151551738C>T]
1030-1	NM	Typical	11.5 (a)	c.[3252_3255+3del];[(11289+1_11290-1)_(11601+1_11602-1)del]	p.[(?)];[(?)]	g.[151679718_151679724del];[(151612390_151614275)_(151614588_151616001)del]
1077-1	NM	Typical	5 (a)	c.[8889+1G>A];[2943G>A]	p.[(?)];[Glu981=)]	g.[151639856C>T];[151682662C>T]
1077-4	NM	Typical	1.75 (a)	c.[8889+1G>A];[2943G>A]	p.[(?)];[Glu981=)]	g.[151639856C>T];[151682662C>T]
1081-1	NM	Mild	55 (a)	c.[13147C>T];[?]	p.[(Gln4383Ter)];[?]	g.[151603685G>A];[?]
1082-1	NM	Typical	18 (a)	c.[3879+1G>A];[8425C>T]	p.[(?)];[Arg2809Ter)]	g.[151675286C>T];[151640615G>A]
1082-4	NM	Typical	13 (a)	c.[3879+1G>A];[8425C>T]	p.[(?)];[Arg2809Ter)]	g.[151675286C>T];[151640615G>A]
1088-1	NM	Severe	0.1 (d)	c.[3390T>G];[3255+3_3255+4insTT]	p.[(Tyr1130Ter)];[(?)]	g.[151678053A>C];[151679717_151679718insAA]
1092-1	NM	Typical	49 (a)	c.[18261+1G>T];[18261+1G>T]	p.[(?)];[(?)]	g.[151565715C>A];[151565715C>A]
1135-1	NM	Intermediate	1.5 (a)	c.[9619-2A>G];[24473_24476dup]	p.[(?)];[His8159GlnfsTer8)]	g.[151630821T>C];[151496965_151496968dup]
1160-1	NM	Typical	5 (a)	c.[17654G>A];[24771del]	p.[(Trp5885Ter)];[(Phe8257LeufsTer10)]	g.[151568398C>T];[151493783del]
1213-1	NM[13]	Intermediate	0.8 (a)	c.[7431+1919_7536+374del];[7431+1919_7536+374del]	p.[(?)];[(?)]	g.[151645758_151648259del];[151645758_151648259del]
1236-1	NM	Typical	10 (a)	c.[24380_24383dup];[6385C>T]	p.[(His8128GlnfsTer8)];[Gln2129Ter)]	g.[151497650_151497653dup];[151656263G>A]
1269-1	NM	Intermediate	5 (a)	c.[20657dup];[3879+1G>T]	p.[(Arg6887AlafsTer13)];[(?)]	g.[151541473dup];[151675286C>A]
1293-1	NM	Typical	14 (a)	c.[24218C>A];[13059+5G>A]	p.[(Ser8073Ter)];[(?)]	g.[151499299G>T];[151604555C>T]
1295-1	NM	Intermediate	3.75 (a)	c.[5343+5G>A];[17654G>A]	p.[(?)];[Trp5885Ter)]	g.[151664754C>T];[151568398C>T]
1302-1	NM	Intermediate	5.75 (a)	c.[23136dup];[24771del]	p.[(Asp7713ArgfsTer13)];[Phe8257LeufsTer10)]	g.[151514416dup];[151493783del]
1304-1	CFTD	Typical	7 (a)	c.[(4506+1_4507-1)_(16908+1_16909-1)del];[?]	p.[(?)];[?]	g.[(151575800_151576150)_(151669132_151671022)del];[?]
1392-1	NM	Severe	8 (a)	c.[3255+1G>A];[1896+2T>C]	p.[(?)];[(?)]	g.[151679720C>T];[151694321A>G]
1413-1	NM	Mild	12 (a)	c.[3987+1_3987+2delinsTG];[989C>G]	p.[(?)];[Thr330Arg)]	g.[151674475_151674476delinsCA];[151709702G>C]
1426-1	NM	Mild	30 (a)	c.[9619-2A>G];[?]	p.[(?)];[?]	g.[151630821T>C];[?]
1427-1	NM	Adult	54 (a)	c.[24219+1G>A];[?]	p.[(?)];[?]	g.[151499297C>T];[?]
1431-1	NM	Typical	4.5 (a)	c.[7431+1919_7536+374del];[?]	p.[(?)];[?]	g.[151645758_151648259del];[?]

eTable 3. Patients with variants in the *NEB* gene (Cont'd).

ID	Dx[a]	Severity[b]	Age (Status)[c]	Genotype (NM_001271208.2)	Predicted proteins (NP_001258137.2)	Genomic Variants (NC_000002.12)
1440-1	NM	Typical	9 (a)	c.[5244_5254delinsG]; [24770_24771dup]	p.[(Tyr1749AsnfsTer23)]; [Ser8258LeufsTer10)]	g.[151664848_151664858delinsC]; [151493782_151493783dup]
1513-1	NM	Mild	42 (a)	c.[4602del];[?]	p.[(Asn1534LysfsTer2)];[?]	g.[151669036del];[?]

[a]Referring clinicopathological diagnosis and previous publications. "NM" = nemaline myopathy, "CFTD" = congenital fiber type disproportion,

[b]Clinical severity according to the 2000 NM classification scheme. "IRM" = Intranuclear Rod Myopathy, "Opht" = Other form with ophthalmoplegia, "Dist" = Other form with unusual distribution of weakness.

[c]Age in years at last known status. "a" = alive, "d" = deceased.

Previous publications with individually identifiable information for subjects.

1. De La Vega FM, Chowdhury S, Moore B, et al. Artificial intelligence enables comprehensive genome interpretation and nomination of candidate diagnoses for rare genetic diseases. Genome Med 2021;13:153.
2. Anderson SL, Ekstein J, Donnelly MC, et al. Nemaline myopathy in the Ashkenazi Jewish population is caused by a deletion in the nebulin gene. Hum Genet 2004;115:185-190.
3. Lehtokari VL, Greenleaf RS, DeChene ET, et al. The exon 55 deletion in the nebulin gene--one single founder mutation with world-wide occurrence. Neuromuscul Disord 2009;19:179-181.
4. Ottenheijm CA, Witt CC, Stienen GJ, Labeit S, Beggs AH, Granzier H. Thin filament length dysregulation contributes to muscle weakness in nemaline myopathy patients with nebulin deficiency. Hum Mol Genet 2009;18:2359-2369.
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6. Lehtokari VL, Pelin K, Sandbacka M, et al. Identification of 45 novel mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. Hum Mutat 2006;27:946-956.
7. Sanoudou D, Corbett MA, Han M, et al. Skeletal muscle repair in a mouse model of nemaline myopathy. Hum Mol Genet 2006;15:2603-2612.
8. Winter JM, Joureau B, Lee EJ, et al. Mutation-specific effects on thin filament length in thin filament myopathy. Ann Neurol 2016;79:959-969.
9. Lawlor MW, Ottenheijm CA, Lehtokari VL, et al. Novel mutations in NEB cause abnormal nebulin expression and markedly impaired muscle force generation in severe nemaline myopathy. Skelet Muscle 2011;1:23.
10. Lehtokari VL, Kiiski K, Sandaradura SA, et al. Mutation update: the spectra of nebulin variants and associated myopathies. Hum Mutat 2014;35:1418-1426.
11. de Winter JM, Buck D, Hidalgo C, et al. Troponin activator augments muscle force in nemaline myopathy patients with nebulin mutations. J Med Genet 2013;50:383-392.
12. de Winter JM, Joureau B, Sequeira V, et al. Effect of levosimendan on the contractility of muscle fibers from nemaline myopathy patients with mutations in the nebulin gene. Skelet Muscle 2015;5:12.
13. Amburgey K, Acker M, Saeed S, et al. A Cross-Sectional Study of Nemaline Myopathy. Neurology 2021;96:e1425-e1436.

eTable 4. Patients with variants in the *CFL2*, *KLHL40*, *KLHL41*, *LMOD3*, *TNNT1*, *TPM2* or *TPM3* genes.

ID	Dx[a]	Severity[b]	Age (Status)[c]	Genotype	Predicted proteins	Genomic Variants (GRCh38)
Individuals with *CFL2* Variants (NM_138638.5, NP_619579.1, NC_000014.9)						
106-1	NM[1]	Typical	2.25 (a)	c.[103G>A];[103G>A]	p.[(Ala35Thr)];[(Ala35Thr)]	g.[34713462C>T];[34713462C>T]
106-2	NM[1]	Typical	9 (a)	c.[103G>A];[103G>A]	p.[(Ala35Thr)];[(Ala35Thr)]	g.[34713462C>T];[34713462C>T]
Individuals with *KLHL40* Variants (NM_152393.4, NP_689606.2, NC_000003.12)						
74-1	NM[2, 3]	Severe	0.08 (d)	c.[100G>C];[257T>C]	p.[(Asp34His)];[(Leu86Pro)]	g.[42685718G>C];[42685875T>C]
74-4	NM[2, 4]	Severe	0 (d)	c.[100G>C];[257T>C]	p.[(Asp34His)];[(Leu86Pro)]	g.[42685718G>C];[42685875T>C]
1191-1	NM[2]	Severe	0.1 (a)	c.[270C>G];[270C>G]	p.[(Tyr90Ter)];[(Tyr90Ter)]	g.[42685888C>G];[42685888C>G]
Individuals with *KLHL41* Variants (NM_006063.3,NP_006054.2, NC_000002.12)						
203-1	NM[5]	Intermediate	16 (a)	c.[103T>C];[103T>C]	p.[(Cys35Arg)];[(Cys35Arg)]	g.[169509881T>C];[169509881T>C]
832-1	NM[4, 5]	Typical	17 (a)	c.[459delinsACTC];[459delinsACTC]	p.[(Ser153_Ala154insLeu)];[(Ser153_Ala154insLeu)]	g.[169510237delinsACTC];[169510237delinsACTC]
Individuals with *LMOD3* Variants (NM_198271.5, NP_938012.2, NC_000003.12)						
869-1	NM	Severe	0.1 (d)	c.[1099_1100del];[1099_1100del]	p.[(Asn367GlnfsTer11)];[(Asn367GlnfsTer11)]	g.[69119256_69119257del];[69119256_69119257del]
1107-1	NM	Typical	17 (a)	c.[1103_1105del];[131_132del]	p.[(Asn368del)];[(Pro44ArgfsTer15)]	g.[69119254_69119256del];[69122255_69122256del]
1120-1	NM[3, 6]	Severe	0.1 (d)	c.[1099_1100del];[1099_1100del]	p.[(Asn367GlnfsTer11)];[(Asn367GlnfsTer11)]	g.[69119256_69119257del];[69119256_69119257del]
1120-5	NM[6]	Severe	0.002 (d)	c.[1099_1100del];[1099_1100del]	p.[(Asn367GlnfsTer11)];[(Asn367GlnfsTer11)]	g.[69119256_69119257del];[69119256_69119257del]
1266-1	NM	Typical	9 (a)	c.[920G>C];[920G>C]	p.[(Arg307Pro)];[(Arg307Pro)]	g.[69119435C>G];[69119435C>G]
Individuals with *TNNT1* Variants (NM_003283.6, NP_003274.3, NC_000019.10)						
38-1	NM[7, 8]	Mild	66 (a)	c.[311A>T];[311=]	p.[(Glu104Val)];[(Glu104=)]	g.[55140959T>A];[55140959=]
148-1	MmD	Typical	36 (a)	c.[287T>C];[287T>C]	p.[(Leu96Pro)][(Leu96Pro)]	g.[55141208A>G];[55141208A>G]
305-1	NM[9]	Typical	25 (a)	c.[215A>T];[215=]	p.[(Glu72Val)];[(Glu72=)]	g.[55141280T>A];[55141280=]
506-1	NM	Adult/IRM	60 (d)	c.[244A>G];[244=]	p.[(Ile82Val)];[(Ile82=)]	g.[55141251T>C];[55141251=]
1193-1	NCM	Typical	21 (a)	c.[191A>C];[191=]	p.[(Asp64Ala)];[(Asp64=)]	g.[55141858T>G];[55141858=]
1299-1	NCM	Severe	1.25 (a)	c.[328_331del];[328_331del]	p.[(Arg110ProfsTer23)];[(Arg110ProfsTer23)]	g.[55140943_55140946del];[55140943_55140946del]
1410-1	MmD[10]	Intermediate	8 (a)	c.[287T>C];[287T>C]	p.[(Leu96Pro)][(Leu96Pro)]	g.[55141208A>G];[55141208A>G]

eTable 4. Patients with variants in the *CFL2*, *KLHL40*, *KLHL41*, *LMOD3*, *TNNT1*, *TPM2* or *TPM3* genes (Cont'd).

ID	Dx[a]	Severity[b]	Age (Status)[c]	Genotype	Predicted proteins	Genomic Variants (GRCh38)
Individuals with *TPM2* Variants (NM_003289.4, NP_003280.2, NC_000009.12)						
75-4	NM	Mild	8 (a)	c.[20_22del];[20_22=]	p.[(Lys7del)];[(Lys7=)]	g.[35689803_35689805del];[35689796_35689798=]
1112-1	NM	Typical	14 (a)	c.[606C>A];[606=]	p.[(Asn202Lys)];[(Asn202=)]	g.[35684765G>T];[35684765=]
1227-1	NM	Typical	23 (a)	c.[20_22del];[20_22=]	p.[(Lys7del)];[(Lys7=)]	g.[35689803_35689805del];[35689796_35689798=]
1227-3	NM	Typical	54 (a)	c.[20_22del];[20_22=]	p.[(Lys7del)];[(Lys7=)]	g.[35689803_35689805del];[35689796_35689798=]
1227-5	NM	Typical	58 (a)	c.[20_22del];[20_22=]	p.[(Lys7del)];[(Lys7=)]	g.[35689803_35689805del];[35689796_35689798=]
1349-1	CFTD	Typical	10 (a)	c.[415_417del];[415_417=]	p.[(Glu139del)];[(Glu139=)]	g.[35685512_35685514del];[35685509_35685511=]
1360-1	NM[11]	Intermediate	8 (a)	c.[5A>T];[5=]	p.[(Asp2Val)];[(Asp2=)]	g.[35689813T>A];[35689813=]
1428-1	Cap M[11]	Intermediate	7 (a)	c.[121G>A];[121=]	p.[(Glu41Lys)];[(Glu41=)]	g.[35689265C>T];[35689265=]
1428-2	Cap M[11, 12]	Typical	45 (a)	c.[121G>A];[121=]	p.[(Glu41Lys)];[(Glu41=)]	g.[35689265C>T];[35689265=]
Individuals with *TPM3* Variants (NM_152263.4, NP_689476.2, NC_000001.11)						
13-2	NM[13, 14]	Intermediate	12 (a)	c.[855-1G>A];[857A>C]	p.[(?)];[(Ter286SerextTer57)]	g.[154167941C>T];[154167938T>G]
126-1	CFTD[15, 16]	Typical	22 (a)	c.[11C>T];[11=]	p.[(Ala4Val)];[(Ala4=)]	g.[154192008G>A];[154192008=]
126-2	CFTD[15, 16]	Mild	39 (a)	c.[11C>T];[11=]	p.[(Ala4Val)];[(Ala4=)]	g.[154192008G>A];[154192008=]
135-1	NM	Intermediate	0.8 (d)	c.[117+2_117+5del];[117+164C>T]	p.[(?)];[(?)]	g.[154191900_154191903del];[154191738G>A]
135-2	NM[3]	Intermediate	4 (d)	c.[117+2_117+5del];[117+164C>T]	p.[(?)];[(?)]	g.[154191900_154191903del];[154191738G>A]
247-4	CFTD[15, 16]	Typical	18 (a)	c.[503G>A];[503=]	p.[(Arg168His)];[(Arg168=)]	g.[154172971C>T];[154172971=]
311-1	CFTD[14-17]	Typical	17 (a)	c.[721G>A];[721=]	p.[(Glu241Lys)];[(Glu241=)]	g.[154170454C>T];[154170454=]
313-1	CFTD[14-16]	Intermediate	19 (a)	c.[857A>C];[857A>C]	p.[(Ter286SerextTer57)];[(Ter286SerextTer57)	g.[154167938T>G];[154167938T>G]
343-1	NM[14-16]	Typical	63 (d)	c.[503G>A];[503=]	p.[(Arg168His)];[(Arg168=)]	g.[154172971C>T];[154172971=]
859-1	NM	Mild	3.75 (a)	c.[495+1_495+5del];[495+1_495+5=]	p.[(?)];[(?)]	g.[154173081_154173085del];[154173079_154173083=]
913-1	CFTD[14-17]	Intermediate	6 (a)	c.[272G>C];[272=]	p.[(Arg91Pro)];[(Arg91=)]	g.[154176220C>G];[154176220=]
1109-1	CFTD[16]	Typical	36 (a)	c.[502C>T];[502=]	p.[(Arg168Cys)];[(Arg168=)]	g.[154172972G>A];[154172972=]
1149-1	CFTD	Typical	8 (a)	c.[502C>T];[502=]	p.[(Arg168Cys)];[(Arg168=)]	g.[154172972G>A];[154172972=]

eTable 4. Patients with variants in the *CFL2*, *KLHL40*, *KLHL41*, *LMOD3*, *TNNT1*, *TPM2* or *TPM3* genes (Cont'd).

ID	Dx[a]	Severity[b]	Age (Status)[c]	Genotype	Predicted proteins	Genomic Variants (GRCh38)
1197-1	CFTD	Typical	5 (a)	c.[503G>A];[503=]	p.[(Arg168His)];[(Arg168=)]	g.[154172971C>T];[154172971=]
1260-1	Cap M	Typical	9 (a)	c.[502C>G];[502=]	p.[(Arg168Gly)];[(Arg168=)]	g.[154172972G>C];[154172972=]
1280-1	NCM	Typical	11 (a)	c.[503G>A];[503=]	p.[(Arg168His)];[(Arg168=)]	g.[154172971C>T];[154172971=]
1346-1	NM	Typical	1.5 (a)	c.[117G>A];[117G>A]	p.[(Gln39=)];[(Gln39=)]	g.[154191902C>T];[154191902C>T]
1455-1	CFTD	Typical	27 (a)	c.[502C>T];[502=]	p.[(Arg168Cys)];[(Arg168=)]	g.[154172972G>A];[154172972=]

[a]Referring clinicopathological diagnosis and previous publications. "NM" = nemaline myopathy, "MmD" = multiminicore disease, "NCM" = nonspecific congenital myopathy, "CFTD" = congenital fiber type disproportion, "Cap M" = cap myopathy.
[b]Clinical severity according to the 2000 NM classification scheme. "IRM" = Intranuclear Rod Myopathy.
[c]Age in years at last known status. "a" = alive, "d" = deceased.

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eTable 5. Statistical Analyses and Software Packages[a]

Odds ratios of clinical findings and long-term outcomes	Fisher's exact test	GraphPad Prism 9.1.0 (216) for Mac, GraphPad Software, San Diego, California USA (https://www.graphpad.com)
Amburgey Clinical Severity Scores	Kruskal-Wallis analysis with Dunn's correction for multiple comparisons	GraphPad Prism 9.1.0 (216) for Mac, GraphPad Software, San Diego, California USA (https://www.graphpad.com)
Comparison of 2000 NM classification scheme to Sewry NM Classification Scheme	Spearman correlation	GraphPad Prism 9.1.0 (216) for Mac, GraphPad Software, San Diego, California USA (https://www.graphpad.com)
Survival Analyses (Fig 2)	Kaplan-Meier analysis using log-rank test, adjusted for multiple testing using Benjamini-Hochberg correction	R Project for Statistical Computing and Package for Survival analysis in R, version 3.2-7.
Comparison of Survival times	Pairwise comparisons using Log- Rank test with Benjamini- Hochberg correction to adjust for multiple testing	R Project for Statistical Computing and Package for Survival analysis in R, version 3.2-7.
Odds of placing into the original severity class based on birth respiratory distress or hypotonia at birth	Fisher's exact test and ordinal logistic regression	R Project for Statistical Computing and Package for Survival analysis in R, version 3.2-7

[a]Description of types of analyses and statistical programs used for analysis of results.

eTable 6: Clinical characteristics by 2000 NM classification scheme category

	Severe	Intermediate	Typical	Mild	Adult onset	Other features
Total patients n (%)	63 (23)	59 (21)	117 (42)	24 (9)	12 (4)	56
Presence of Rods/# patients had biopsy in severity group						
Yes	42/61	41/56	63/101	14/20	10/11	40/50
Fracture and Contractures						
Birth Contractures	34	8	3	0	0	7
Birth Fractures	15	1	1	0	0	1
Scoliosis	11	32	28	5	0	40
Scoliosis Surgery	3	14	9	3	0	8
Motor Delay/patients who survived past and had evaluation after 1.5 yrs of age						
Yes	15/18	43/45	84/108	3/24	0/12	25/47
Unknown	3/18	0/45	9/108	4/24	8/12	11/47
Speech Delay/patients who survived past and had evaluation after 1.5 yrs of age						
Yes	8/18	16/45	23/108	1/24	0/12	6/47
Unknown	6/18	5/45	15/108	5/24	8/12	12/47
Respiratory Status						
Invasive ventilation	43	27	3	0	0	5
>22hr PPV	0	2	1	0	0	0
night-time PPV	4	8	21	4	0	3
other	3	10	6	0	1	4
none	4	11	86	20	10	33
unknown	6	0	0	0	0	1
total	60	58	117	24	11	46
Feeding Status						
GT/NG	43	43	18	0	1	11
other	2	0	0	0	0	0
support later removed	2	3	16	0	0	2
none	3	13	81	24	10	32
unknown	6	0	2	0	1	2
total	56	59	117	24	11	47

Clinical cohort characteristics by 2000 NM classification scheme (Wallgren-Pettersson & Laing, 2000). Severity was unable to be classified in 1 fetus. “Other features” included cardiomyopathy, ophthalmoplegia, unusual distribution of weakness, and intranuclear nemaline bodies. Patients with this classification also received a severity score 1-5 (severe, intermediate, typical/congenital, mild/juvenile or adult onset). Totals of respiratory status and feeding status excludes terminations and stillbirths, as well as one patient who passed away before feedings were attempted.

eTable 7: Inheritance by genotype

	NEB	*ACTA1*	*TPM2*	*TPM3*	*TNNT1*	*KLHL40*	*KLHL41*	*LMOD3*	*CFL2*
Total cases (n=220)	95	79	9	18	7	3	2	5	2
AR or presumed AR[a]	65	2	9	5	3	3	2	5	2
AD or presumed AD	1	77	0	13	4	0	0	0	0
De novo variant[b]	1	26	0	3	0	0	0	0	0
Questioned mosaicism[b]	0	2	0	0	0	0	0	0	0
Missing allele	29	0	0	0	0	0	0	0	0

Inheritance patterns by genotype. AR = autosomal recessive, AD = autosomal dominant. [a] Includes no/incomplete phasing of some biallelic cases, [b] included in AD totals.

eTable 8: Clinical characteristics by gene

	NEB	*ACTA1*	*TPM2*	*TPM3*	*TNNT1*	*KLHL40*	*KLHL41*	*LMOD3*	*CFL2*
Total patients n,(%)	95 (35)	79 (29)	9 (3)	18 (7)	7 (2)	3 (1)	2 (0.5)	5 (1)	2 (0.5)
Severity (2000 NM Classification Scheme)									
Severe congenital	8	26	0	0	1	3	0	3	0
Intermediate	27	15	2	5	1	0	1	0	0
Typical	48	32	6	11	3	0	1	2	2
Mild	10	5	1	2	1	0	0	0	0
Adult onset	2	1	0	0	1	0	0	0	0
Presence of Rods/# patients that had biopsy in gene group									
Yes	78/84	40/69	3/5	7/18	3/7	2/3	2/2	3/4	2/2
Orthopedic Issues									
Birth Contractures	5	12	1	0	4	2	0	3	0
Birth Fractures	0	7	0	0	0	2	0	0	0
Scoliosis	32	16	3	9	4	0	1	1	0
Scoliosis Surgery	13	7	2	3	1	0	1	0	0
Motor Delay/patients who survived past and had evaluation after 1.5 yrs of age									
Yes	68/87	49/54	6/9	13/17	2/6	0/1	2/2	2/2	2/2
Unknown	3/87	7/54	2/9	3/17	1/6	0/1	0/2	0/2	0/2
Ambulation/patients who survived past and had evaluation after 1.5 yrs of age									
Yes	52/87	19/54	6/9	10/17	3/6	0/0	1/2	2/2	2/2
Never gained	19/87	33/54	1/9	6/17	3/6	0/0	1/2	0/2	0/2
Gained but lost later	15/87	2/54	2/9	0/17	0/6	0/0	0/2	0/2	0/2
Unknown	1/87	0/54	0/9	1/17	0/6	0/0	0/2	0/2	0/2
Speech Delay/patients who survived past and had evaluation after 1.5 yrs of age									
Yes	25/87	15/54	2/9	2/17	4/6	0/0	1/2	0/2	1/2
Unknown	9/87	15/54	3/9	2/17	1/6	0/0	0/2	1/2	0/2

eTable 8: Clinical characteristics by gene (Cont'd)

	NEB	*ACTA1*	*TPM2*	*TPM3*	*TNNT1*	*KLHL40*	*KLHL41*	*LMOD3*	*CFL2*
Respiratory Status									
Invasive ventilation	22	27	0	2	0	3	1	4	0
>22hr PPV	2	1	0	0	0	0	0	0	0
Night-time PPV	13	10	2	7	4	0	0	0	0
Other	8	7	1	1	0	0	0	0	0
None	49	33	6	7	3	0	1	1	2
Unknown	0	1	0	1	0	0	0	0	0
Total patients[a]	95	79	9	18	7	3	2	5	2
Feeding Status									
GT/NG	35	45	0	2	0	3	1	2	0
Other	0	1	0	0	0	0	0	1	0
Support later removed	8	7	1	0	0	0	0	1	0
None	49	26	8	16	7	0	1	1	2
Unknown	2	0	0	0	0	0	0	0	0
Total patients	95	79	9	18	7	3	2	5	2

Totals of respiratory status and feeding status excludes terminations and stillbirths, as well as one patient who passed away before feedings were attempted.

eTable 9: Neurological findings

	Total Cohort	*NEB*	*ACTA1*	*TPM2*	*TPM3*	*TNNT1*	*KLHL40*	*KLHL41*	*LMOD3*	*CFL2*	No molecular dx
EMG abnormal	N=78/132	27/51	25/33	1/3	6/12	1/5	0	1/2	1/2	1/1	15/23
Neurogenic EMG findings	N=21 (17% of EMG cohort)	7/51	5/32	0/3	2/12	0/5	0	1/2	0/2	0/1	6/23
Myopathic EMG findings	N=61 (48% of EMG cohort)	17/51	17/32	1/3	5/12	1/5	0	0/2	1/2	1/1	18/23
Unusual weakness distribution	N=36/219	17/83	7/63	1/8	2/18	1/5	0/3	0/2	0/3	0/1	8/33
Absent reflexes	N=74/208	29/78	26/61	2/7	10/16	1/3	2/3	1/2	0/2	0/2	4/34
Decreased reflexes	N=106/208	44/78	26/61	4/7	5/16	2/3	1/3	1/2	2/2	2/2	19/34
Cognitive deficits	N=10/200	4/84	3/49	0/8	0/18	1/7	NA	0/2	0/2	0/2	2/28
Learning disability	N=10/200	6/84	2/49	0/8	1/18	0/7	NA	0/2	0/2	0/2	1/28
Elevated CK (>450)	N=16/116	1/36	6/40	1/4	0/9	2/2	0/2	0/1	0/1	1/2	5/19

Neurological findings in the cohort. EMG reports were not available for 13 of these cases. Unusual distribution of weakness included asymmetric or distal>proximal weakness. Number of cases by genotype for each finding are represented as the fraction of total informative cases for each genotype/clinical finding comparison.

eTable 10: Histopathological findings

	Number cases with finding / cases with finding specified present or absent									
Category (No. cases w/ bx data)	Nemaline Rods / bodies	Intra-nuclear rods[a]	Caps[a]	Excessive fiber size variation[b]	Type 1 fiber pre-dominance	Type 2 fiber pre-dominance	Type 1 fiber hypotrophy / atrophy[c]	Multi-minicores	Central nuclei	Endomysial fibrosis
Overall (220)[d]	194/220	0	0	166/202	108/158	9/158	16/177	22/204	53/151	60/173
Genotype										
NEB (70)	69/70	0	0	48/63	51/57	1/57	2/52	6/65	10/47	18/57
ACTA1 (58)	48/58	4	0	46/54	22/39	3/39	5/38	4/53	7/37	13/47
TPM2 (5)	4/5	0	1	5/5	3/4	1/4	1/5	1/5	2/5	0/3
TPM3 (16)	6/16	0	1	15/16	6/6	0/6	8/15	2/16	7/14	5/13
TNNT1 dom (2)	1/1	0	0	2/2	1/2	0/2	0/1	0/1	2/3	0/1
TNNT1 rec (3)	2/2	0	0	3/3	1/2	0/2	0/3	0/2	1/2	1/2
KLHL40 (2)	2/2	0	0	0/1	1/1	0/1	0/2	0/2	1/2	2/2
KLHL41 (2)	2/2	0	0	1/2	2/2	0/2	0/1	0/2	0/2	0/2
LMOD3 (4)	4/4	0	0	3/4	2/4	1/4	0/3	0/3	1/1	2/4
CFL2 (2)	1/2	0	0	2/2	1/2	0/2	0/2	1/2	2/2	0/2
Clinical severity										
Severe (55)	50/55	2	0	41/49	19/36	6/36	2/47	2/50	10/35	17/43
Intermediate (49)	44/49	3	0	41/43	29/37	0/37	3/42	6/46	8/32	10/36
Typical (87)	73/87	1	2	62/82	51/67	3/67	10/67	9/81	22/64	23/71
Mild (17)	15/17	0	0	13/17	7/12	0/12	1/14	4/15	9/12	4/14
Adult onset (11)	11/11	0	0	8/10	2/6	0/6	0/7	1/11	4/7	5/8

[a]Nuclear rods and caps reported without denominators as these findings are rarely mentioned when absent.
[b]Reported as “moderate” or greater.
[c]Represents likely underestimate as fiber type distribution of disproportionate fibers is often not specified.
[d]Includes 56 cases of unknown genotype and 1 terminated pregnancy of unknown severity.

eTable 11: Characteristics of adult-onset cases

ID	Genotype[a]	Age of onset (yrs)	Presenting symptom	Distribution of weakness	Time to status (yrs)	Status	Serum CK (U/L)[b]	Diagnostic findings
144-1	WES-neg	30-40	Rt foot drop	Distal	13	Alive	nl	Rod bodies, fiber size variation, endomysial fibrosis
188-1	*ACTA1*	35-45	Weakness	Proximal	10	Deceased	nl	Rod bodies, fiber size variation
506-1	*TNNT1*	40-50	Proximal weakness	Lft-Rt asymmetry	21	Deceased	nl	Cytoplasmic and nuclear rod bodies, fiber size variation, fibrofatty infiltration
768-1[c,d]	WES-neg	40-50	Proximal weakness	Proximal	2	Deceased	496	Rod bodies, monoclonal gammopathy
791-1[c]	WES-neg	55-65	Back weakness	Proximal, paraspinal	16	Alive	nl	Rod bodies, Type 1 predominance, fibrofatty infiltration
874-1[c]	WES-neg	55-65	Difficulty rising from chair	Proximal	4	Alive	nl	Rod bodies, fiber size variation, fibrofatty infiltration, monoclonal gammopathy
890-1	*NEB*	35-45	Difficulty walking	Lft-Rt asymmetry	9	Alive	nl	Rod bodies, fibrofatty infiltration
936-1[c]	WES-neg	60-70	Neck weakness	Neck only	0	Alive	nl	Rod bodies, mild fiber size variation
1251-1[c]	WES-neg	50-60	Weakness	Unknown	7	Alive	nl	Rod bodies, fiber size variation, Type 1 predominance, fibrofatty infiltration
1291-1[c]	WES-neg	35-45	Shortness of breath, arm pain	Proximal	0	Alive	1232	Rod bodies, fiber size variation
1386-1[c]	WES-neg	45-55	Proximal leg weakness	Arm strength preserved	10	Alive	635, 592	Rod bodies, fiber size variation, monoclonal gammopathy excluded
1427-1	*NEB*	40-50	Proximal weakness, elevated palate	Proximal	7	Alive	nl	Not done

[a] Details in eTables 2-4. "WES-neg" = no pathogenic variants identified on short-read whole exome sequencing.
[b] "nl" serum creatine kinase defined as < 200 U/L.
[c] Meets criteria for diagnosis of Sporadic Late Onset Nemaline Myopathy (SLONM).
[d] Previously reported by Keller CE, Hays AP, Rowland LP, et al., Adult-onset nemaline myopathy and monoclonal gammopathy. Arch Neurol 2006;63:132-134.