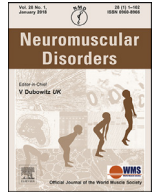




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Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

A cross-sectional study in 18 patients with typical and mild forms of nemaline myopathy in the Netherlands

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ARTICLE INFO

Article history:

Received 6 March 2024

Revised 15 July 2024

Accepted 1 August 2024

Keywords:

Nemaline myopathy

Typical form

Mild form

Trial readiness

Cohort

ABSTRACT

Nemaline myopathy (NM) is a congenital myopathy with generalised muscle weakness, most pronounced in neck flexor, bulbar and respiratory muscles. The aim of this cross-sectional study was to assess the Dutch NM patient cohort. We assessed medical history, physical examination, quality of life (QoL), fatigue severity, motor function (MFM), and respiratory muscle function. We included 18 of the 28 identified patients (13 females (11–67 years old); five males (31–74 years old)) with typical or mild NM and eight different genotypes. Nine patients (50 %) used a wheelchair, eight patients (44 %) used mechanical ventilation, and four patients (22 %) were on tube feeding. Spinal deformities were found in 14 patients (78 %). The median Medical Research Council (MRC) sum score was 38/60 [interquartile range 32–51] in typical and 48/60 [44–50] in mild NM. The experienced QoL was lower and fatigue severity was higher than reference values of the healthy population. The total MFM score was 55 % [49–94] in typical and 88 % [72–93] in mild NM. Most of the patients who performed spirometry had a restrictive lung function pattern (11/15). This identification and characterisation of the Dutch NM patient cohort is important for international collaboration and can guide the design of future clinical trials.

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1. Introduction

Nemaline myopathy (NM) is a congenital myopathy with characteristic nemaline rods in muscle biopsy with a clinical

manifestation ranging from mild to severe NM [1]. The prevalence estimate of the all-age population is 0.2 per 100,000 [2]. NM may present prenatally with polyhydramnios and/or weak or infrequent foetal movements, at birth in the form of a “floppy infant”, later in childhood, or even in adulthood [3]. The muscle weakness is usually generalised and most pronounced in the neck flexors and bulbar muscles in NM in general [4,5]. Most patients suffer from weakness of the respiratory muscles, which may be more

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severely affected than other muscles, and respiratory insufficiency is the most common cause of death [3,5,6]. Many patients need mechanical ventilation and mobility aids [5]. Scoliosis can be a common clinical sign, appearing mostly in the prepubertal period of rapid growth, often at the thoracolumbar junction [3].

Causative variants in at least 14 genes have been identified including the *NEB*, *ACTA1*, *CFL2*, *TPM2*, *TPM3*, *KLHL40*, *KLHL41*, *TNNT1*, *TNNT3*, *RYR1*, *RYR3*, *KBTD13*, *MYPN*, and *LMOD3* genes [7–21]. *NEB* and *ACTA1* variants are the most prevalent worldwide [1,22]. The recently revised classification distinguishes six forms of NM based on the age of onset and severity [1]: (1) severe NM (contractures, fractures, no respiratory effort or no movements at birth); (2) typical NM (perinatal onset and motor milestones delayed but reached); (3) mild NM (childhood or juvenile onset); (4) distal NM (distal weakness); (5) childhood-onset NM with slowness, or nemaline myopathy type 6 (NEM6) (slowness of movements and core-rod histology, caused by the *KBTD13* variant [23–25]); and (6) recessive *TNNT1* (former Amish) NM.

Clinical characterisation of cohorts of patients with NM is essential to identify the limitations of patients with this rare disease and to provide suitable therapies [3,5]. Moreover, the 2019 European Neuromuscular Center (ENMC) international workshop on trial readiness in NM suggested that an international registry to collect data on large cohorts of patients is important for trial readiness [26]. Cohort studies are also important for subsequent natural history studies to identify suitable outcome measures for future clinical trials. Thus far, no cohort study has been performed in Dutch patients with NM, except for our cohort of patients with NEM6 [25].

This is the first cross-sectional cohort study that systematically performed a clinical characterisation of the Dutch cohort of patients with NM. Moreover, this study assessed quality of life (QoL) and fatigue in patients with NM in contrast to previously performed studies. The broad range of data obtained in our cohort enabled us to provide suggestions on future clinical trials.

2. Patients and methods

2.1. Study design and patients

This cross-sectional study was performed between October 2018 and September 2020 at the Radboud university medical center, Nijmegen, the Netherlands. Ethical approval (NL65214.091.18 & protocol 2017-4022) was granted by the regional ethics committee and written informed consent was given by all patients or legal representatives. Patients were invited to the outpatient clinic for a single visit. Those who declined a visit were offered a home visit using the same study protocol, except for spirometry.

Patients with NM were identified through five routes: (1) (paediatric) neurology outpatient clinic at the Radboud university medical center; (2) (paediatric) neurologists and rehabilitation specialists of neuromuscular centers in the Netherlands; (3) the Dutch patient organisation for neuromuscular diseases (Spierziekten Nederland); (4) pulmonologists/internists/paediatricians of the four Dutch centers for Home Mechanical Ventilation and (5) relatives with NM. The inclusion criteria were a known causative genetic variant of NM or the combination of an NM clinical phenotype, a biopsy confirming NM, and a first-degree family member with a causative genetic variant of NM. The age range was six up to 80 years. By excluding younger and older patients we aimed to increase the likelihood for patients to be cognitively and physically able to carry out the tests. We excluded patients with NEM6. Since childhood-onset NM with slowness or NEM6 is a distinct phenotype and has a milder clinical severity than most other forms of NM, we will report these patients separately.

2.2. Medical history and physical examination

The form of NM was determined according to the recently revised classification distinguishing six forms of NM based on the age of onset and severity [1]: (1) severe NM (contractures, fractures, no respiratory effort or no movements at birth); (2) typical NM (perinatal onset and motor milestones delayed but reached); (3) mild NM (childhood or juvenile onset); (4) distal NM (distal weakness); (5) childhood-onset NM with slowness, or NM type 6 (NEM6) (slowness of movements and core-rod histology, caused by the *KBTD13* variant [23–25]); and (6) recessive *TNNT1* (former Amish) NM. Perinatal and current symptoms were systematically assessed from the electronic patient records and by assessing the medical history. A systematic physical examination was conducted by one investigator/clinician (EK). This included manual muscle testing (Medical Research Council (MRC) scores) [27] of neck flexors and extensors, shoulder abductors, elbow flexors and extensors, wrist flexors and extensors, finger abductors, hip flexors and extensors, knee flexors and extensors; and foot dorsiflexors and plantar flexors. The MRC sum score consisted of the sum of MRC scores of six individual muscle groups bilaterally: shoulder abductors, elbow flexors, wrist extensors, hip flexors, knee extensors and foot dorsiflexors, with a maximum score of 60 [28]. Facial muscle weakness was classified as muscle weakness of (one of the) facial muscles and/or the presence of a myopathic facies. The presence of skeletal abnormalities was examined, i.e. high-arched palate, hyperlordosis, scoliosis (Adam's forward bend test [29]), and joint contractures.

2.3. Questionnaires

Standardised questionnaires were used in adult patients only ($n = 15$). The short form health survey (SF-36) questionnaire (Dutch version 2.0) was used to assess the QoL from nine aspects: physical functioning (10 items), bodily pain (2 items), role limitations due to physical health problems (4 items), role limitations due to personal or emotional problems (3 items), emotional well-being (5 items), social functioning (2 items), energy/fatigue (4 items), general health perceptions (5 items), and perceived change in health (1 item) [30,31]. It contains 36 items, and the concepts are scored on a 0–100 % range, with 100 % being the most favourable state of health. The results were compared with a commonly used representative sample of the healthy Dutch population [32], and populations with facioscapulohumeral muscular dystrophy (FSHD), laminin $\alpha 2$ (LAMA2)-related muscular dystrophy and selenoprotein N (SELENON)-related congenital myopathy [33–36].

Fatigue severity was assessed by the checklist individual strength (CIS) [37,38]. This 20-item questionnaire contains four subscales: perceived fatigue severity (8 items), concentration (5 items), motivation (4 items), and physical activity (3 items). The items are scored on 7-point Likert scales; hence the sum score ranges from 20 to 140. A higher score is indicative of a higher disease burden. Problematic fatigue is defined as a total score of ≥ 76 [39], and severe experienced fatigue as a fatigue severity subscale score of ≥ 35 [40]. A representative sample of the healthy Dutch general population was used as a reference group [40] and we also compared the results with the abovementioned patient populations with neuromuscular disorders.

2.4. Motor function measure

The 32-item motor function measure (MFM (third edition, 2009)) consists of three different domains; (1) standing position and transfers (scored 0–39); (2) axial and proximal motor function (scored 0–36); and (3) distal motor function (scored 0–21) [41].

The total score (0–96) is expressed as a percentage of the maximum score, and a lower score indicates lower motor function.

2.5. Spirometry and respiratory muscle strength testing

As respiratory muscle weakness is an important and frequent feature of NM and is an essential part of the clinical characterisation, we included previously published data on respiratory muscle function from the same cohort [6]. Spirometry was carried out with a handheld electronic spirometer (SpiroUSB, Vyair Medical, Mettawa, IL, USA connected to PC Spirometry software, Spida CareFusion 2.3.0.10 for Windows 7). Forced vital capacity (FVC) and vital capacity (VC) upright and supine, as well as forced expiratory volume in the first second (FEV1) were measured and compared with reference values [42]. Peak cough flow (PCF) was also measured. Respiratory muscle strength testing, including maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), and sniff nasal inspiratory pressure (SNIP), was performed with a handheld electronic manometer (Micro RPM, Micro Medical, Rhymney, UK connected to Puma software version 1.4.2) and compared with reference values [43,44]. Both spirometry and respiratory muscle strength tests were performed in accordance with the standards of the American Thoracic Society and the European Respiratory Society [45–47].

2.6. Data analysis

The data was stored in Castor EDC (Castor clinical data management platform, Amsterdam, The Netherlands). GraphPad Prism software version 9.5.0 (GraphPad Software, San Diego, CA, USA), and SPSS (version 27, IBM, Armonk, New York) was used for the statistical analysis and visualisation.

Results are expressed as median [interquartile range (IQR)] or mean (standard deviation (SD)). One-sample *t*-tests were used to compare the scores of the SF-36 and the CIS questionnaires with the reference values for the healthy population, FSHD, LAMA2, and SELENON group. Mann-Whitney U tests compared the MFM scores between patients with the different NM forms. Spearman's rho tests were used to assess the correlation between the total MFM score and FVC% predicted. A *p*-value <0.05 was considered to represent statistical significance.

3. Results

3.1. Patient characteristics

We identified a total of 64 patients with NM (Fig. 1A), their genotypes are shown in Fig. 1B. After excluding patients with a pathogenic variant in the *KBTD13* gene (NEM6), 28 patients were contacted (17 females and 11 males). Three patients were excluded because their genotype was unknown; four paediatric patients and two adult patients declined to participate because they considered the study to be too strenuous or time consuming; and one patient did not want to participate because of the presumed risk of contracting a SARS-CoV-2 infection. Finally, eighteen patients from 14 families with eight different genotypes (Fig. 1C) participated in our study, including 13 female (72 %; aged 11 to 67 years) and 5 male patients (28 %; aged 31 to 74 years). Three of the female patients were children. Patient characteristics are displayed in Table 1. We included two sisters with a *TPM2* genotype, two sisters with a *RYR1* genotype, a brother and sister with a *NEB* genotype, and a father and son with an *ACTA1* genotype. One of the sisters with the *RYR1* genotype showed nemaline rods in the muscle biopsy and these sisters could therefore be included in our study. Three patients were visited at home. NM was diagnosed by genetic testing in 17 patients, of whom 15 initially underwent a muscle

Table 1
Patient characteristics.

| | |
|-------------------------------|------------------|
| Number of patients | 18 |
| Age (median years [IQR]) | 29.5 [18.0–62.3] |
| Sex (n) | 13 F, 5 M |
| BMI (kg/m ² [IQR]) | 21.5 [17.9–25.0] |
| Age of onset (years [IQR]) | 0 [0–6] |
| Phenotype (n(%)) | |
| Typical | 11 (61 %) |
| Mild | 7 (39 %) |
| Genotype (n(%)) | |
| <i>CFL2</i> | 1 (6 %) |
| <i>LMOD3</i> | 1 (6 %) |
| <i>MYPN</i> | 1 (6 %) |
| <i>TPM3</i> | 1 (6 %) |
| <i>RYR1</i> | 2 (11 %) |
| <i>TPM2</i> | 2 (11 %) |
| <i>ACTA1</i> | 5 (28 %) |
| <i>NEB</i> | 5 (28 %) |

IQR, interquartile range. BMI, body mass index.

biopsy. One patient had a clinical and histological phenotype consistent with NM, and a first-degree family member with a *NEB* genotype. Eleven patients had the typical NM form and seven patients had the mild form. Supplementary Table 1 shows the individual patient data on genotypes, ambulatory status, cardiac symptoms, need for a feeding tube and ventilatory status.

3.2. Medical history

The medical history is presented in Table 2 with a subdivision between patients with the typical and mild NM form. Most patients in both groups experienced functional difficulty or inability in performing daily activities, which was also reflected by the high prevalence of limitations in job or school activities. Nine patients (50 %) with the typical form used a wheelchair (seven powered, two manual) of whom five (28 %) were wheelchair dependent. The initial age of wheelchair use is shown in Fig. 2.

Table 2
Medical history.

| | Total number of patients n = 18 | Typical NM form n = 11 | Mild NM form n = 7 |
|--|------------------------------------|---------------------------|-----------------------|
| Perinatal symptoms | | | |
| Decreased foetal movements | 9 (50 %) | 8 (73 %) | 1 (14 %) |
| Hypotonia | 10 (56 %) | 10 (91 %) | 0 (0 %) |
| Respiratory insufficiency | 6 (33 %) | 6 (55 %) | 0 (0 %) |
| Delayed motor milestones | 13 (72 %) | 11 (100 %) | 2 (29 %) |
| Feeding tube | 7 (39 %) | 6 (55 %) | 1 (14 %) |
| Functional difficulties or inability in | | | |
| Washing | 14 (78 %) | 9 (82 %) | 5 (71 %) |
| Dressing | 14 (78 %) | 8 (73 %) | 6 (86 %) |
| Driving a car (n/total n (%)) | 11/15 (73 %) | 8/9 (89 %) | 3/6 (50 %) |
| Climbing stairs | 17 (94 %) | 11 (100 %) | 6 (86 %) |
| Cycling | 17 (94 %) | 10 (91 %) | 7 (100 %) |
| Running | 18 (100 %) | 11 (100 %) | 7 (100 %) |
| Ambulatory status | | | |
| No assistance | 4 (22 %) | 2 (18 %) | 2 (29 %) |
| Walking aids | 5 (28 %) | 0 (0 %) | 5 (71 %) |
| Wheelchair assistance | 4 (22 %) | 4 (36 %) | 0 (0 %) |
| Wheelchair dependent | 5 (28 %) | 5 (46 %) | 0 (0 %) |
| Job/school affected | | | |
| Job affected (n/total n (%)) | 4/6 (67 %) | 2/3 (67 %) | 2/3 (67 %) |
| (Partially) declared unfit for work | 9/12 (75 %) | 4/6 (67 %) | 5/6 (83 %) |
| School affected (n/total n (%)) | 6/7 (86 %) | 6/6 (100 %) | 0/1 (0 %) |
| Special education | 2/7 (29 %) | 2/6 (33 %) | 0/1 (0 %) |
| Adaptations at school | 4/7 (57 %) | 4/6 (67 %) | 0/1 (0 %) |
| Performing sports | 7 (39 %) | 3 (27 %) | 4 (57 %) |

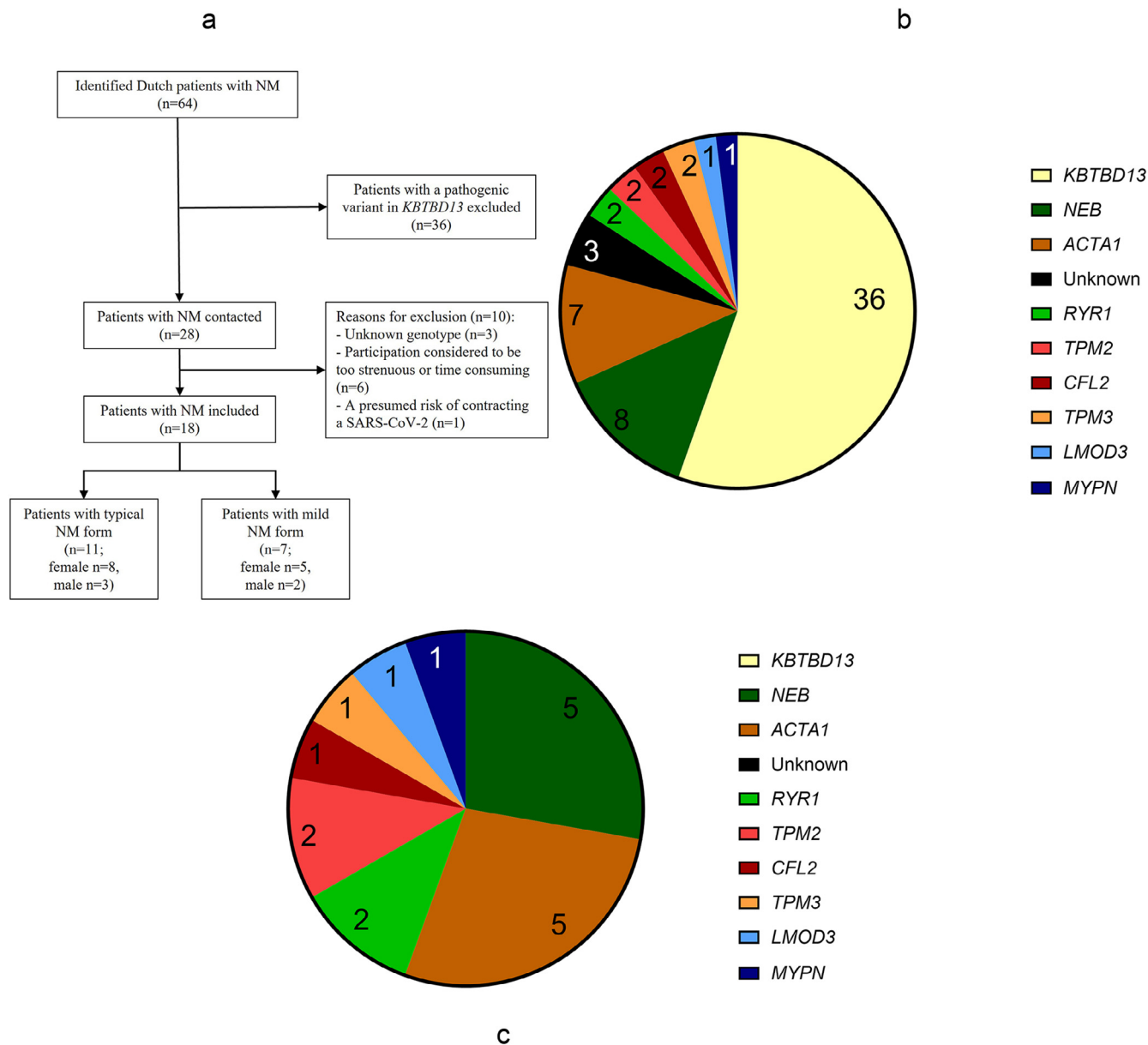


Fig. 1. Patient inclusion. The figure shows (A) The flowchart of patient inclusion (B) Total number of genotypes of all identified patients and (C) Total number of genotypes of the participants in this study.

Walking aids were used by five patients (28 %) with the mild NM form and included a cane ($n = 2$) or a walker ($n = 3$).

The reported non-skeletal muscle manifestations and comorbidities in this cohort of patients with NM are displayed in Table 3. There were five patients with cardiac manifestations or cardiac comorbidities, including one patient with a cardiomyopathy and one patient with cardiac arrhythmia. Four patients with typical NM used a feeding tube (three patients with a percutaneous radiologic gastrostomy tube; one patient with a percutaneous endoscopic gastrostomy tube). A mode of mechanical ventilation was used by 11 patients (61 %), of which nine patients used non-invasive mechanical ventilation and two patients with typical NM used invasive mechanical ventilation. The age at initiating (non-)invasive mechanical ventilation use is shown in Fig. 2. A high prevalence of disturbed sleep was reported ($n = 9$; 50 %). Most patients reported that the cause was related to mechanical ventilation ($n = 5$; 28 %). Type 2 diabetes,

hypertension, hypercholesterolemia, and breast cancer were not more common in this cohort than in the general population in relation to age.

3.3. Physical examination

The median MRC sum score was 38 [32–51] out of 60 in patients with the typical NM form and 48 [44–50] out of 60 in the mild NM form (Fig. 3A). The mean of the individual muscles included in the MRC sum score was 3.3 (± 0.80) for the typical NM form and 3.9 (± 0.39) for the mild NM form. The median MRC scores for individual muscles are shown in Fig. 3B. Table 4 shows the results of the tests assessing muscle involvement and skeletal abnormalities. Most patients showed facial muscle weakness ($n = 16$; 89 %). Spinal deformities were found in 14 patients (78 %), for which five patients had undergone scoliosis surgery.

Table 3
Reported non-skeletal muscle manifestations and comorbidities.

| | Total number of patients n = 18 | Typical NM form n = 11 | Mild NM form n = 7 |
|------------------------------------|------------------------------------|---------------------------|-----------------------|
| Cardiac symptoms | | | |
| Cardiomyopathy | 1 (6 %) | 1 (9 %) | 0 (0 %) |
| Cardiac arrhythmia | 1 (6 %) | 0 (0 %) | 1 (14 %) |
| Myocardial infarction | 1 (6 %) | 1 (9 %) | 0 (0 %) |
| Palpitations | 2 (11 %) | 2 (18 %) | 0 (0 %) |
| Gastrointestinal symptoms | | | |
| Irritable bowel syndrome | 1 (6 %) | 0 (0 %) | 1 (14 %) |
| Cholelithiasis | 1 (6 %) | 0 (0 %) | 1 (14 %) |
| Diarrhoea | 3 (17 %) | 1 (9 %) | 2 (29 %) |
| Feeding tube | 4 (22 %) | 4 (36 %) | 0 (0 %) |
| Obstipation | 4 (22 %) | 3 (27 %) | 1 (14 %) |
| Pulmonary symptoms | | | |
| Lower respiratory tract infections | 6 (33 %) | 5 (45 %) | 1 (14 %) |
| Shortness of breath | 14 (78 %) | 10 (91 %) | 4 (57 %) |
| Mechanical ventilation | | | |
| Nocturnal non-invasive | 8 (44 %) | 7 (64 %) | 1 (14 %) |
| Nocturnal and daytime non-invasive | 1 (6 %) | 0 (0 %) | 1 (14 %) |
| Invasive by tracheostomy | 2 (11 %) | 2 (18 %) | 0 (0 %) |
| Skeletal symptoms | | | |
| Bone fractures | 4 (22 %) | 2 (18 %) | 2 (29 %) |
| Other | | | |
| Type 2 diabetes | 4 (22 %) | 1 (9 %) | 3 (43 %) |
| Hypertension | 4 (22 %) | 2 (18 %) | 2 (29 %) |
| Hypercholesterolemia | 4 (22 %) | 2 (18 %) | 2 (29 %) |
| Urolithiasis | 1 (6 %) | 1 (9 %) | 0 (0 %) |
| Breast cancer | 2 (11 %) | 0 (0 %) | 2 (29 %) |
| Attention Deficit Disorder | 1 (6 %) | 0 (0 %) | 1 (14 %) |
| Disturbed sleep | 9 (50 %) | 4 (36 %) | 5 (71 %) |

3.4. QoL and fatigue

On the SF-36 questionnaire assessed in adults, patients scored significantly less favourable state of health scores than healthy subjects on physical functioning, limitations due to physical and emotional health problems, emotional well-being, energy/fatigue, and general health (Table 5). Compared with patients with other neuromuscular disorders, patients with NM scored similar health scores.

Perceived fatigue and the total score on the CIS, assessed in adult patients, were significantly higher in comparison with the reference group, reflecting a larger disease burden. The

Table 4
Results of physical examination.

| | Total number of patients n = 18 | Typical NM form n = 11 | Mild NM form n = 7 |
|--------------------------------|------------------------------------|---------------------------|-----------------------|
| Muscle involvement | | | |
| Weakness of the facial muscles | 16 (89 %) | 11 (100 %) | 5 (71 %) |
| Myopathic face | 11 (61 %) | 8 (73 %) | 3 (43 %) |
| Walking on toes | 11 (61 %) | 8 (73 %) | 3 (43 %) |
| Walking on heels | 14 (78 %) | 10 (91 %) | 4 (57 %) |
| Skeletal abnormalities | | | |
| Scoliosis/kyphosis/lordosis | 14 (78 %) | 10 (91 %) | 4 (57 %) |
| Scoliosis surgery | 5 (28 %) | 4 (36 %) | 1 (14 %) |
| Contractures | 10 (56 %) | 7 (64 %) | 3 (43 %) |
| High-arched palate | 15 (83 %) | 11 (100 %) | 4 (57 %) |

scores were comparable to patients with other neuromuscular disorders (Table 5). Five patients (45 %) with the typical, and five patients (71 %) with the mild NM form had scores indicating severe experienced fatigue. Problematic fatigue was found in three patients (27 %) with the typical and five patients (71 %) with the mild NM form.

3.5. Motor function measure

Fig. 4 shows the results of the total score on the MFM and the separate domains. The median score on the total MFM score was 55 % [49–94] and 88 % [72–93], on domain 1 15 % [8–92] and 77 % [54–95], on domain 2 69 % [61–92] and 89 % [86–100], and on domain 3 95 % [86–100] and 95 % [95–100], in the typical and mild NM form, respectively. There were no significant differences between NM forms for the separate domains and the total MFM score.

3.6. Spirometry and respiratory muscle strength testing

The results from spirometry and respiratory muscle strength testing are shown in Table 6. Spirometry was obtained from 15 patients who visited the hospital, i.e. 10 patients with a typical NM form and five patients with a mild NM form. The PCF result of one patient with a typical form is missing. Most patients (except one patient with typical NM and two with a mild form) had an FVC below 80 % of the predicted value. FEV1/FVC was above 70 % in all patients, thus most patients with NM had a restrictive

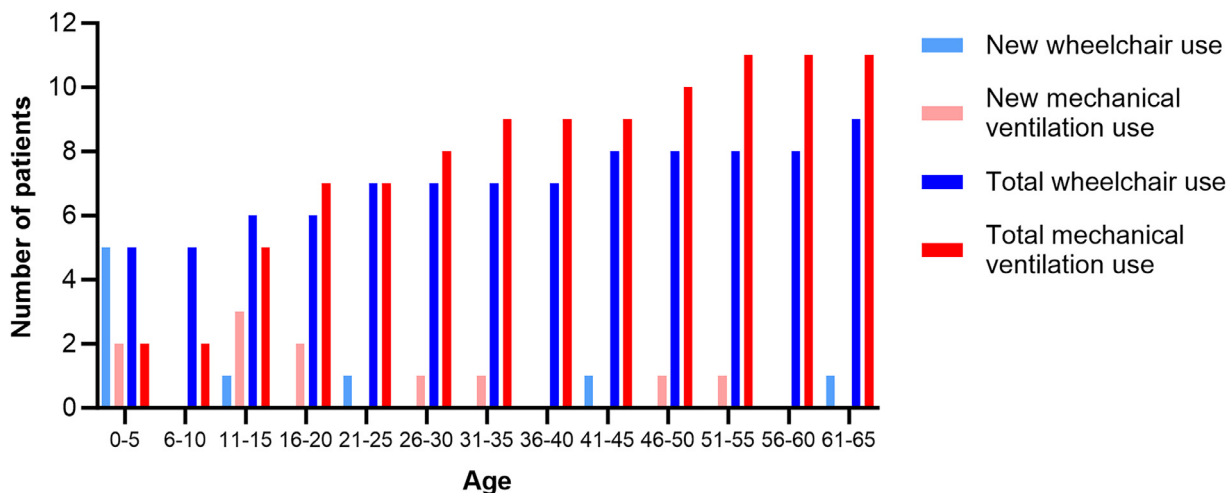


Fig. 2. Initial age of wheelchair (n = 9/18) and mechanical ventilation (n = 11/18) use. The figure shows the number of patients that use a wheelchair and/or (non)-invasive mechanical ventilation. It is indicated when patients started using a wheelchair/mechanical ventilation (new wheelchair/mechanical ventilation use). The total number of patients using a wheelchair/mechanical ventilation at different age ranges is also shown (total wheelchair/ mechanical ventilation use).

Table 5
Results of SF-36 and CIS questionnaires in adults.

| | Scores of adult patients with NM | Scores of reference group of healthy subjects | Scores of FSHD patients | Scores of LAMA2 patients | Scores of SELENON patients |
|--|----------------------------------|---|-------------------------|--------------------------|----------------------------|
| SF-36 (% of total score) | n = 15 | n = 1063 | n = 139 | n = 15 | n = 5 |
| Physical functioning | 33.0 (29.1) | 81.9 (23.2)* | 45.2 (31.4) | 15.0 (24.9) | 15.0 (11.7) |
| Bodily pain | 78.9 (26.4) | 79.5 (25.6) | 66.6 (23.8) | 90.2 (13.1) | 72.2 (22.0) |
| Limitations due to physical health problems | 58.1 (30.2) | 79.4 (35.5)* | 47.9 (42.0) | 73.3 (35.9) | 25.0 (35.4) |
| Limitations due to emotional health problems | 68.3 (22.7) | 84.1 (32.2)* | 69.5 (41.6) | 88.9 (27.2)* | 40.0 (54.8) |
| Emotional well-being | 65.3 (18.4) | 76.8 (18.4)* | 72.6 (17.0) | 88.3 (9.4)* | 80.0 (13.0) |
| Social functioning | 74.3 (31.4) | 86.9 (20.5) | 71.6 (24.2) | 84.2 (21.4) | 82.5 (25.9) |
| Energy/fatigue | 45.1 (23.7) | 67.4 (19.9)* | – | 67.7 (17.0)* | 52.0 (10.4) |
| General health | 44.7 (19.4) | 72.7 (22.7)* | 51.7 (21.6) | 47.3 (27.4) | 38.0 (16.4) |
| Perceived change in health | 56.7 (22.1) | 52.4 (19.4) | – | – | – |
| CIS | n = 15 | n = 1923 | n = 373 | n = 15 | n = 5 |
| Perceived fatigue (range 8–56) | 37.3 (12.6) | 23.0 (10.75)* | 37.0 (12.0) | 31.6 (5.2) | 34.6 (3.1) |
| N ≥ 35 | 10 (56 %) | – | – | – | – |
| Total score (range 20–140) | 80.5 (27.1) | 54.8 (21.48)* | 76.0 (24.0) | 76.3 (12.8) | 80.2 (3.4) |
| N ≥ 76 | 8 (44 %) | – | – | – | – |

The means of the 36-Item Short Form Health Survey (SF-36) questionnaire are compared to a healthy reference group[32], FSHD patients[34], LAMA2 patients[35] and SELENON patients[36]. The standard deviation is between parentheses. Higher scores are a more favourable health state. The means of the Checklist Individual Strength (CIS) are compared to another healthy reference group[40], FSHD patients[33], LAMA2 patients[35] and SELENON patients[36]. A higher score is indicative of a higher disease burden. * Significant difference (p-value<0.05) in comparison to NM. NM, nemaline myopathy. FSHD, facioscapulohumeral muscular dystrophy. LAMA2, laminin α2-related muscular dystrophy. SELENON, selenoprotein N-related congenital myopathy.

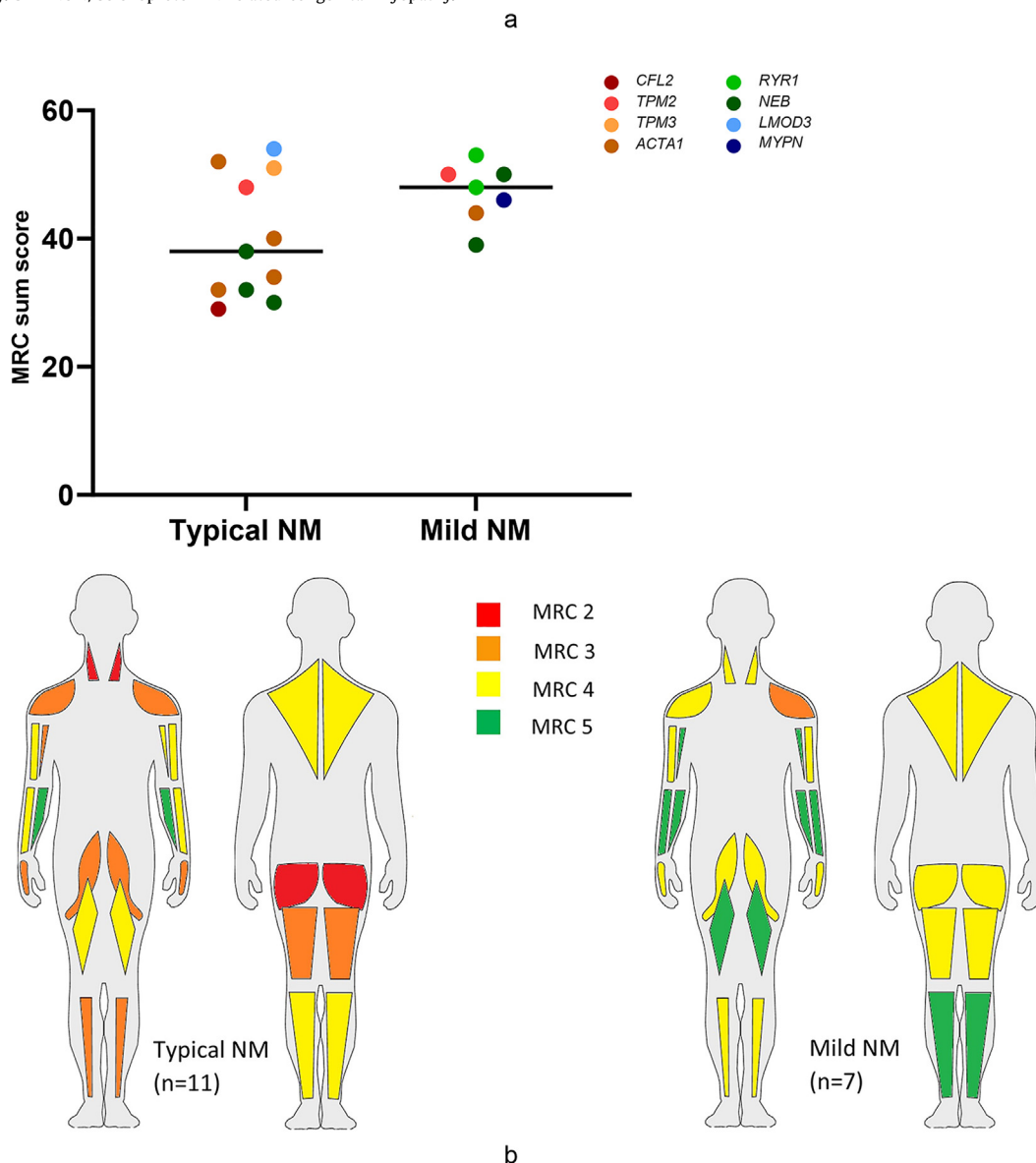


Fig. 3. A: MRC sum scores depicted for the typical (n = 11) and mild (n = 7) form of NM. The dots represent individual patients and the black line the median. The different colours represent different genotypes. Fig. 3B: Median MRC scores for individual muscles depicted for the typical (n = 11) and mild (n = 7) form of NM.

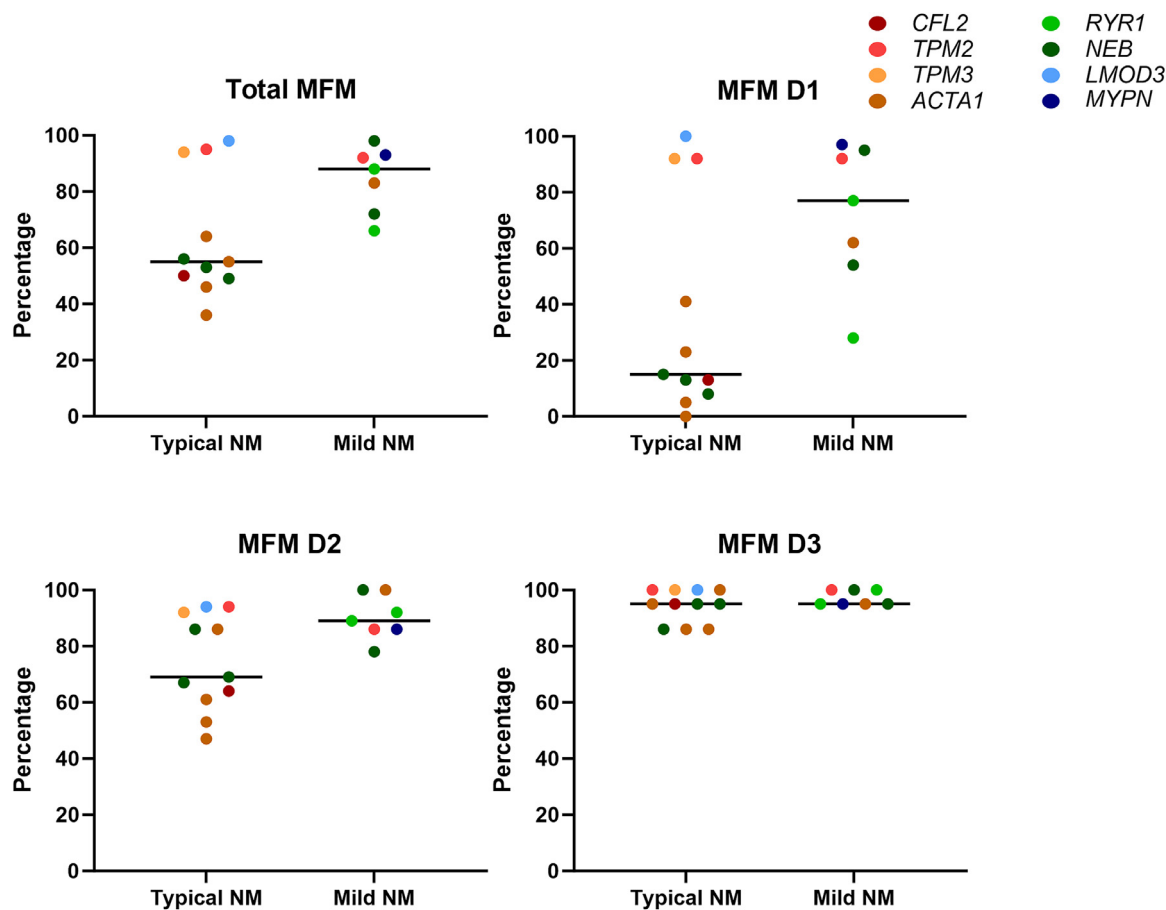


Fig. 4. MFM score percentages depicted for the typical ($n = 11$) and mild ($n = 7$) form of NM. The figures show the total MFM score and the 3 domains (D1: standing position and transfers, D2: axial and proximal motor function, D3: distal motor function). The patients are divided by form of NM and colour coded by genotype. The black lines are the median. No statistically significant differences were found between the NM forms. MFM, Motor Function Measure.

Table 6
Results on spirometry and respiratory muscle strength testing.

| | Total number of patients | Typical NM form | Mild NM form |
|--|----------------------------|----------------------------|---------------------------|
| Spirometry | $n = 15$ | $n = 10$ | $n = 5$ |
| FVC (L) | 2.0 [1.0–2.4] | 1.1 [1.0–2.3] | 2.3 [1.8–2.9] |
| FVC(% predicted) | 48 [31.0–76.0] | 32.0 [29.0–58.5] | 69.5 [46.3–82.5] |
| VC (L) | 1.9 [1.0–2.4] | 1.1 [0.9–2.2] | 2.2 [1.7–2.9] |
| Change VC upright and supine (%) | –3.0 [–16– –1.0] | –9.0 [–18.8– –1.3] | –2.0 [–10.8– –0.5] |
| FEV1/FVC | 88.0 [77.0–91.0] | 90.0 [82.0–95.0] | 80.5 [72.5–88.8] |
| PCF (L/min) | 230.5 [135.8– 389.8] | 179.5 [101.3–307.3] | 324.5 [217.5–428.8] |
| Respiratory muscle strength testing | $n = 18$ | $n = 11$ | $n = 7$ |
| MIP (cmH ₂ O) | 41 [24.8–79.8] | 35 [24.0–55.0] | 68 [35.0–92.0] |
| MIP (% predicted) | 54 [37.3–103.8] | 50 [32.0–77.0] | 103 [42.0–122.0] |
| MEP (cmH ₂ O) | 53 [27.0–82.3] | 36 [26.0–63.0] | 80 [63.0–123.0] |
| MEP (% predicted) | 42 [31.5–74.5] | 35 [28.0–46.0] | 79 [42.0–135.0] |
| SNIP(cmH ₂ O) | 50 [19.5–63.5] | 35[18.8–60.3] | 55 [50.0–83.0] |
| SNIP (% predicted) | 54 [20.0–65.0] | 36 [19.5–63.5] | 59 [48.0–102.0] |

The spirometry results are obtained from patients who visited the hospital, i.e. 10 patients with typical and 5 patient with mild NM. The PCF result of one patient with the typical form is missing. Data are presented as median [IQR]. These results were published earlier[6]. NM, nemaline myopathy. FVC, forced vital capacity. VC, vital capacity. FEV1, forced expiratory volume in first second. PCF, peak cough flow. MIP, maximal inspiratory pressure. MEP, maximal expiratory pressure. SNIP, sniff nasal inspiratory pressure. IQR, interquartile range.

lung function pattern. This is also in line with respiratory muscle strength testing as most median results are below predicted values. There was a strong correlation between total MFM scores and FVC% predicted ($r(13)=0.763, p < .001$).

4. Discussion

In this cross-sectional cohort study, we systematically assessed the overall clinical phenotype of the Dutch cohort of patients

with NM. We extensively assessed the medical history, physical examination, QoL, fatigue severity, motor function, and respiratory muscle function. Patients ($n = 18$) with a broad spectrum of genotypes and ages were included with either a typical or mild NM form. The main findings were: (1) half of the patients need a wheelchair; (2) the majority of the patients have spinal deformities; (3) there is a low experienced QoL; (4) there is a high experienced fatigue severity; (5) the majority of the patients have a restrictive lung function pattern and use mechanical

ventilation; (6) there is a wide variation in motor function, except for a relatively spared distal motor function; and (7) there is a strong correlation between motor function and respiratory function.

Comparison between patients with the typical and mild NM form showed more wheelchair assistance and dependence in the typical NM form. Physical examination showed lower MRC scores and more skeletal abnormalities, especially spinal deformities, in patients with the typical NM form compared with the mild form. However, median scores on domain 1 (standing position and transfers) and total MFM score between the groups did not show a significant difference, possibly due to the relatively low number of patients.

Interestingly, we observed a high clinical heterogeneity. In a family with an *ACTA1* genotype and a family with a *TPM2* genotype, one family member had a typical NM form and the other a mild form. This is in line with previous findings in patients with a *NEB* genotype, as there was no statistically significant genotype–phenotype correlation [48]. Moreover, the same *NEB* variant was associated with different forms of NM. A study comparing phenotypes of patients with *NEB* genotypes with those of patients with *ACTA1* genotypes reported scarce genotype–phenotype correlations, as there was a large overlap in phenotypes between these genotypes [4]. However, the course of the disease was generally milder in patients with a *NEB* genotype. Another study found more feeding support in patients with *ACTA1* variants in comparison with patients with *NEB* variants [5]. A study on the genotype–phenotype correlations in patients with *TPM2* and *TPM3* variants found that NM caused by *TPM2* variants usually have a milder presentation than NM caused by *TPM3* variants [49]. However, no clear correlation between the type of variant and the phenotype was found. Thus, overall, there is a weak genotype–phenotype correlation in NM.

The lower QoL score, higher prevalence of experienced fatigue, and lesser social participation are likely to be related to the functional limitations in daily life activities. The low social participation was reflected in the inability of children to attend schools without adaptations and in adults with limitations in performing a paid job. Interestingly, this was not reflected by lower scores on social functioning in the SF-36 in adults. The perceived change in health on the QoL questionnaire was not different from observations in healthy subjects, which probably is related to the fact that NM often follows a relatively non-progressive course [5]. The similar results on QoL and fatigue in the different neuromuscular disorders (FSHD, LAMA2-related muscular dystrophy, and SELENON-related congenital myopathy) show that the burden of disease is equivalent.

In our cohort, impaired respiratory function, use of tube feeding, and skeletal deformities were frequently present, especially in patients with the typical NM form. In our previously published extensive evaluation of respiratory muscle function of the full Dutch NM cohort, we found a low correlation between motor function and the degree of respiratory muscle weakness. We now report a strong correlation. This is explained by the fact that patients with NEM6 are excluded in the current publication, as these patients have a relatively preserved respiratory muscle function [6]. We found a low prevalence of comorbidities and there was a low prevalence of cardiac disease specifically. These findings are in line with other NM cohort studies [3,5].

Our clinical characterisation on Dutch patients with NM is one of the few prospective studies that systematically collected data and provides data on QoL and fatigue severity. By providing these findings on mostly adult patients, our study is complementary to the recent cross-sectional study in 57 patients (median age

8.6 years) with NM recruited at a North American NM family conference [5].

Our results lead to several recommendations for future studies and clinical trials in patients with NM. First, much heterogeneity is found in the phenotypes within the genotypes, thus, based on our study, no subdivision should be made between the genotypes. However, as mentioned earlier there are some specific genotype–phenotype correlations in NM. Thus, based on the type of study, it should be considered making some specific subdivisions based on the genotype. A subdivision based on the form of NM is not ideal either, as the form does not necessarily predict the severity of the disease at a later stage. We suggest that a subdivision based on the severity of the phenotype at time of inclusion is more suitable, for example based on ambulation, use of mechanical ventilation, or the MFM scores. Second, the MFM is an important quantitative functional outcome measure because of the comprehensive assessment of motor function. Third, to identify suitable outcome measures on the experienced low QoL, a focus group meeting could identify the contributing factors. Importantly, in future trials one should consider that treatment policies are different in different countries. This leads to varying patient cohorts and potential difficulties in choosing outcome measures suitable for all.

This study has some limitations. First, we included patients with either a typical or mild form of NM from the age of 11 years or older. Parents of children with a more severe phenotype declined participation because they considered the tests to be too strenuous. Second, we were able to include only one to a few patients for each genotype. With this limited data we were unable to identify specific features for each genotype.

In conclusion, we have provided an extensive clinical characterisation of a cohort of patients with NM in the Netherlands, also assessing QoL and fatigue. The results are important for the NM international registry suggested at the ENMC workshop to collect data on large cohorts of patients with NM for future clinical trials. Moreover, we provide specific suggestions for these trials.

Funding sources

This work was financially supported by the [Prinses Beatrix Spierfonds](#) (Grant No. [W.OR17-08](#)) and A Foundation Building Strength.

Declaration of competing interest

None.

CRediT authorship contribution statement

Esmee S.B. van Kleef: Writing – original draft, Project administration, Methodology, Investigation, Data curation. **Sanne A.J.H. van de Camp:** Writing – review & editing, Visualization, Data curation. **Jan T. Groothuis:** Writing – review & editing. **Corrie E. Erasmus:** Writing – review & editing, Resources. **Michael A. Gaytan:** Writing – review & editing, Resources. **Bettine A.H. Vosse:** Writing – review & editing, Resources. **Willemien de Weerd:** Writing – review & editing, Resources. **Corien C. Verschuuren-Bemelmans:** Writing – review & editing, Resources. **Evita G. Medici-Van den Herik:** Writing – review & editing, Resources. **Carina Wallgren-Pettersson:** Writing – review & editing. **Benno Küsters:** Writing – review & editing. **Meike Schouten:** Writing – review & editing, Resources. **Baziel G.M. van Engelen:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Coen A.C. Ottenheijm:** Writing – review & editing, Methodology,

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Acknowledgments

The patients with nemaline myopathy included in this study are acknowledged for their participation. We would like to thank Erik-Jan Kamsteeg of the department for Human Genetics, Radboudumc, Nijmegen, for his contribution towards diagnosing the patients involved in this study. Several authors of this publication are members of the Radboudumc Center of Expertise for neuromuscular disorders (Radboud-NMD), Netherlands Neuromuscular Center (NL-NMD) and the European Reference Network for rare neuromuscular diseases (EURO-NMD).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.nmd.2024.08.001](https://doi.org/10.1016/j.nmd.2024.08.001).

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