Nemaline Myopathy Type 6 Caused by Variants in the *KBTBD13* Gene

A Cross-Sectional Study of 24 Patients

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Abstract

Background and Objectives
Nemaline myopathy type 6 (NEM6) is the most prevalent type of nemaline myopathy in the Netherlands. Because a detailed clinical characterization is not available yet, we here provide a detailed assessment of 24 patients.

Methods
In this cross-sectional study, we performed a full clinical assessment (medical history and neurologic examination) in patients with NEM6. Patient demographics, causative variants in the *KBTBD13* gene, creatine kinase levels, and the results of previous muscle biopsies were collected. We evaluated experienced health-related quality of life, fatigue severity, prevalence of falls, balance control (Mini-Balance Evaluation Systems Test [Mini-BESTest]), functional motor score (Motor Function Measure [MFM]), and 6-minute walk distance. We used transcranial magnetic stimulation to assess muscle relaxation kinetics.

Results
Twenty-four patients were included (19 women [19–76 years]; 5 men [25–57 years]). Key patient-reported symptoms since childhood were muscle weakness (n = 23; 96%), slowness of movements (n = 23; 96%), and difficulties with running (n = 20; 83%). Axial, proximal, and distal muscles showed mild weakness in most patients. Health-related quality of life was significantly lower, and there was a significantly increased fatigue severity compared with controls. Prospectively, in a period of 100 days, 8 patients (33%) fell at least 1 time, of whom 5 patients (21%) fell 2 times or more. The median total score on the Mini-BESTest was 24 (21.0–26.0 [interquartile range]) of 28 and the median total percentage on the MFM was 91% (83.5–95.3), both considered to be mildly abnormal. The 6-minute walk distance was below the lower limit of normal in 4 patients (17%). All patients with NEM6 showed a markedly reduced muscle relaxation rate with a median of 6.5 [4.9–8.1] s^{-1} (lower limit of normal is 10.1 s^{-1}).

Discussion
This cross-sectional study in patients with NEM6 shows a relatively mild clinical phenotype and mildly abnormal functional tests. However, patients report an important impact on the daily activities, which is illustrated by functional difficulties, reduced quality of life, increased fatigue severity, and increased prevalence of falls. This might be related to delayed muscle relaxation. This study provides a comprehensive overview of the clinical presentation and functional limitations in patients with NEM6.

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Glossary

6MWT = 6-minute walk test; **CIS** = Checklist Individual Strength; **CK** = creatine kinase (CK); **MFM** = Motor Function Measure; **Mini-BESTest** = Mini-Balance Evaluation Systems Test; **MRC** = Medical Research Council; **MVC** = maximal voluntary contraction; **NEM6** = nemaline myopathy type 6; **NpRR** = peak relaxation rate; **SF-36** = 36-Item Short-Form Health Survey; **TMS** = transcranial magnetic stimulation.

Introduction

Nemaline myopathy type 6 (NEM6) is characterized by mild proximal and axial muscle weakness,[1,2] but some patients also have distal muscle weakness.[3,4] The most prominent muscle weakness is in the neck flexors.[1] The most striking patient-reported feature, and distinct from the other forms of NEM, is slowness of movements.[1-3] This is confirmed by an impaired muscle relaxation rate with the use of electrically evoked contractions and transcranial magnetic stimulation (TMS).[5,6] It is important to note that patients can also suffer from cardiomyopathy and respiratory muscle weakness.[7,8] First symptoms are usually noticed around the age of 5 years by gait abnormalities and difficulties performing sports.[3] However, onset at adult age has also been described.[2] Muscle biopsies show nemaline rods and cores or core-like areas due to myofibrillar disorganization.[1,3] NEM6 is the most prevalent type of nemaline myopathy in the Netherlands[9] and was first described in an Australian Dutch family.[1] It is autosomal dominantly inherited and caused by a missense variant in the Kelch repeat and BTB (POZ) domain containing 13 (*KBTBD13*) gene. The c.1222C>T (p.Arg408Cys) is the most prevalent variant in the *KBTBD13* gene in the Netherlands and is also called the Dutch founder variant.[9,10] A limited number of patients are reported with NEM6 worldwide: the Dutch founder variant is also found in a Chinese family[4]; patients with other variants are identified in Spain, Australia, and Italy.[2,3,9]

Patients with NEM6 are not or very sparsely included in large cohort studies on NEM.[11-15] The clinical studies on NEM6 published so far provided a broad overview of the disease including muscle biopsy and imaging results.[1-4] However, a comprehensive cross-sectional study on clinical, functional, and patient-reported outcome measures is lacking. The impact of the disease on the daily lives of patients is unknown. Moreover, combining muscle relaxation experiments with clinical data would help to study the slowness of movements.

In this cross-sectional study, we performed a full clinical and functional assessment in patients with NEM6 and included validated questionnaires (i.e., 36-Item Short-Form Health Survey and Checklist Individual Strength), questions on the prevalence of falls, validated functional tests (i.e., Mini-Balance Evaluation Systems Test [Mini-BESTest], Motor Function Measure [MFM], and 6-minute walk test [6MWT]), and TMS-induced muscle relaxation measurements.

Methods

Patients

We recruited patients with NEM6 from our patient registry and with the help of their relatives with NEM6. We also reached out to clinicians in the 6 neuromuscular centers in the Netherlands. The inclusion criteria were genetically confirmed NEM6 or the combination of a NEM6 clinical phenotype (childhood-onset NEM with slowness of movements),[16] a biopsy confirming NEM6, and a first-degree family member with genetically confirmed NEM6. The age range was 6 to 80 years to increase the likelihood for patients to be cognitively and physically able to perform the tests.

Patient demographics (age, sex, BMI, and age at onset), causative variants in the *KBTBD13* gene, creatine kinase (CK) levels, and the results of muscle biopsies previously published in a histologic characterization study[17] were collected. Additional biopsies were analyzed following a similar protocol.

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained according to the Declaration of Helsinki from all participants. This cross-sectional study was approved by the regional review board *METC Oost-Nederland* (NL65214.091.18).

Study Design and Outcome Measures

This cross-sectional study was performed between October 2018 and June 2020 at the Radboud University Medical Center, Nijmegen, the Netherlands. Patients were invited to the outpatient clinic for 1 visit. In case they were not able or willing to come, they were offered a home visit with the same protocol, except for the 6MWT and TMS measurements.

Perinatal period, childhood, and current symptoms were systematically assessed. Slowness of movements was defined as a slow motor response to sudden unexpected events leading to an inability to perform rapid alternating movements.[1] Muscle stiffness was defined as an experienced stiffness in the muscles not influenced by external factors such as temperature.[1] Moreover, we assessed functional difficulties, the use of assistive devices, and comorbidities.

A systematic physical examination was conducted by one investigator/clinician (E.K.). This included manual muscle testing (Medical Research Council [MRC] scores)[18] of neck flexors, neck extensors, shoulder abductors, elbow flexors, elbow extensors, wrist flexors, wrist extensors, finger abductors,

hip flexors, hip extensors, knee extensors, knee flexors, foot dorsiflexors, and foot plantar flexors. The MRC sum score consisted of the sum of MRC scores of 6 individual muscle groups bilaterally, i.e., shoulder abductors, elbow flexors, wrist extensors, hip flexors, knee extensors, and foot dorsiflexors with a maximum of 60.[19] Facial muscle weakness was classified as muscle weakness of (one of the) facial muscles and/or the presence of a myopathic face. Muscle mass was assessed qualitatively (atrophy, normal, hypertrophy) with special focus on the presence of scapular winging. Gait was assessed qualitatively by evaluating the walking pace, the presence of a waddling gait, a foot drop, or other abnormalities. Furthermore, the presence of skeletal abnormalities was examined, i.e., high-arched palate, hyperlordosis, scoliosis (Adam forward bend test[20]), and joint contractures. Several functional tests were performed, selected based on patient-reported difficulties in previous studies.[1,3] These tests included walking on toes and heels, jumping, climbing stairs, rising from a 30-cm high stool, running, rising from squat position, and hopping. The tests were considered abnormal if the test could not be (fully) performed, if the test was slowly performed, or if there were compensatory movements.

The 36-Item Short-Form Health Survey (SF-36) questionnaire (Dutch version 2.0) was used to assess the quality of life on 8 concepts including physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, general health perceptions, and perceived change in health.[21,22] The questionnaire contains 36 items, and the concepts are scored on a 0–100% range, with 100% being the most favorable health state. The results were compared with a commonly used representative sample of the healthy Dutch population.[23]

Fatigue severity was assessed using the Checklist Individual Strength (CIS).[24,25] This 20-item questionnaire contains 4 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 higher disease burden. Problematic fatigue is defined as a total score of ≥76[26] and severe experienced fatigue as a fatigue severity subscale score of ≥35.[27] A representative sample of the healthy Dutch general population was used as a reference group.[27]

The occurrence of falls was collected retrospectively, defined as a previous fall caused by NEM6, and prospectively for a period of 100 days with a similar protocol as previously used.[28] A fall was defined as an unintended change of posture resulting in coming to rest on the floor, the ground, or another object at a lower level. Moreover, the cause of the fall according to the patient was noted.

The Mini-BESTest was used to assess balance control.[29] This test contains 14 items and covers anticipatory postural adjustments, postural responses, sensory orientation, and stability in gait. The items are scored from 0 (unable or requiring help) to 2 (normal), and the maximum score is 28 points. Patients with a total score of <19 are assumed to have an increased risk of falling.[30]

The 32-item MFM (third edition, 2009) consists of 3 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). The total score (0–96) is expressed as a percentage of the maximum score, and a lower score indicates decreased motor function.

The 6MWT was performed according to the ATS recommendations[31] on a walking course of 25 m, and scores were compared with the lower limits of normal.[32]

The in vivo muscle relaxation rate of the deep finger flexors was assessed through the use of TMS over the motor cortex during maximal voluntary contractions (MVCs). This induces a brief moment of cortical excitation generating a small increase in force followed by an abrupt halt of descending corticospinal drive to the muscle (i.e., the silent period) causing involuntary relaxation of the targeted muscle. We used the same methodology as the one used in previous studies.[33,34] In short, force was measured using strain gauges in a homemade handgrip device. Surface EMG of the deep finger flexors of the dominant hand was recorded to ensure adequate corticospinal suppression during the silent period. Patients performed 3 MVCs with the TMS pulse administered at maximum voluntary force level. Patients were instructed to attempt to continue their active contraction during and after the TMS pulse, allowing for a reliable estimation of the silent period duration. TMS pulses were generated using a Magstim 200 (Magstim, Whitland, United Kingdom) with a 90-mm circular coil positioned over the vertex. The 3 measurements were separated by 1-minute rest to avoid fatigue. Contraindications were epilepsy, pregnancy, metal in the brain or skull, implanted neurostimulator, cardiac pacemaker or intracardiac lines, and medication infusion device.[35,36] Handedness was determined using the Edinburgh Handedness Inventory.[37] The data were processed using a software routine developed in Matlab (Matlab R2014b, The Mathworks, MA). The peak relaxation rate was defined as the steepest negative slope in force during the TMS-induced silent period. This rate was normalized to the force preceding muscle relaxation, resulting in the normalized peak relaxation rate (NpRR). The lower limit of normal (i.e., 5th percentile) for NpRR is 10.1 s^{-1} and 12.0 s^{-1} for women and men, respectively.[33] Patients were excluded if the silent period was <150 ms despite a stimulation intensity of 100% of maximal stimulator output, i.e. 2T.[33] The average of 3 NpRR measurements was calculated, and the highest voluntary MVC was noted. The MVCs were compared with reference values.[38] If patients were not able to participate, previous measurements were used if available.[6] MVC was measured in all patients, except for the home visits.

Table 1 Patient Characteristics

	Number of patients (n = 24)
Age (yrs)	49 [31.0–56.8]
Sex (n)	19 F, 5 M
BMI (kg/m^2)	24.7 [23.5–27.3]
Age at onset (yrs)	7 [6.0–10.0]
Biopsy (n = 8), n (%)	
Cores/core-like areas	7 (88)
Rods	8 (100)
Ring rods (n = 7)	3 (43)
Nuclear clumps	8 (100)
Internalized nuclei	8 (100)
Variant in the KBTBD13 gene (n (%))	
c.1222C>T (p.(Arg408Cys))	23 (96)
c.1222C>A (p.(Arg408Ser))	1 (4)

The medians and interquartile ranges are shown.

Data Analysis

The data were stored in Castor EDC (Castor clinical data management platform, Amsterdam, the Netherlands). GraphPad Prism software version 9.5.0 (GraphPad Software, San Diego, CA) was used for the statistical analysis and visualizations.

Results are expressed as median (interquartile range) or mean (SD). One-sample t-tests were used to compare the scores of the SF-36 and the CIS questionnaires with the reference values. A p value < 0.05 was considered to be statistically significant.

Data Availability

Anonymized data not published within this article will be made available by request from any qualified investigator. Videos of the patients are available on request.

Results

Patients

We identified 26 female adult patients and 10 male adult patients with NEM6. We identified no children between 6 and 18 years. Two patients did not fulfil the inclusion criteria, lacking genetic confirmation of NEM6. Ten patients declined to participate because they found the study too strenuous or time-consuming. Thus, 24 patients participated in our study, including 19 women (79%; aged 19–76 years) and 5 men (21%; aged 25–57 years) (Table 1). Nineteen patients visited our clinic, and 5 were visited at home including 2 women and 3 men. Of these patients, 7 participated in our previous cross-sectional study on the first reported NEM6 family.[1] The

Figure 1 Patient-Reported symptoms
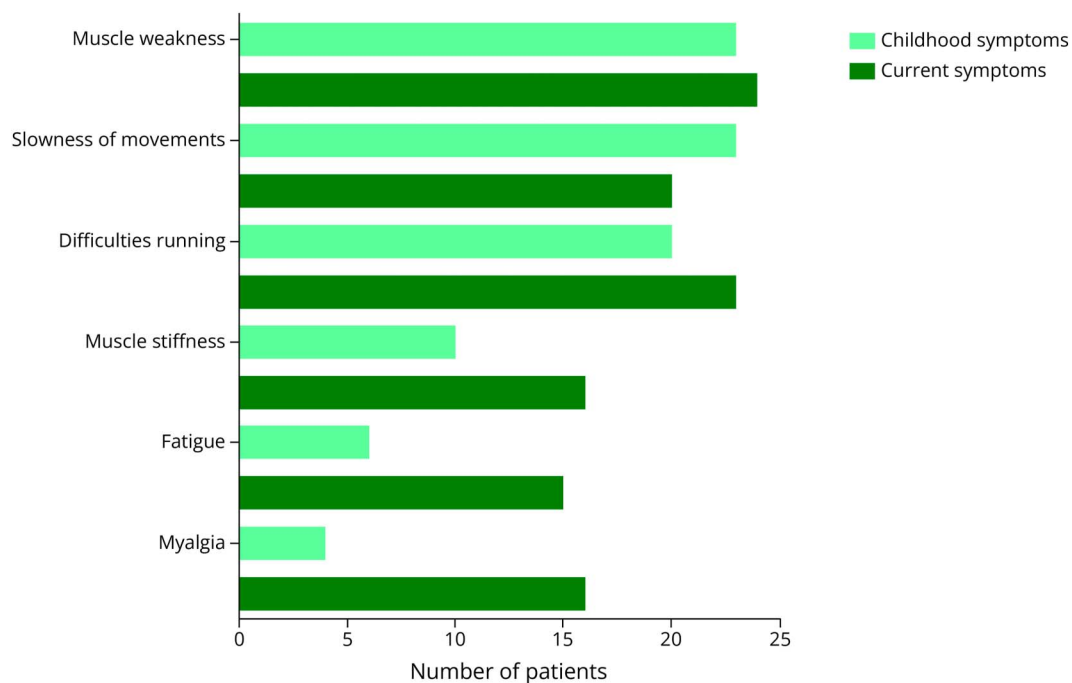
The number of patients reporting this selection of symptoms in childhood or currently. Slowness of movements was defined as a slow motor response to sudden unexpected events and inability to perform rapid movements. Muscle stiffness was defined as a constant experienced stiffness in the muscles not influenced by external factors such as temperature.

histopathologic characteristics of 7 patients were included in our retrospective study,[17] 8 patients were included in our TMS study,[6] and the medical records of 21 patients were used in our study on cardiac dysfunction and cardiomyopathy in NEM6.[7]

In 6 patients, NEM6 was diagnosed by genetic testing and muscle biopsy. In 2 patients, the diagnosis was based on muscle biopsy and a first-degree family member with a *KBTBD13* variant. In 16 patients, only genetic testing was performed. In 14 patients, the CK levels were tested. The mean value was 156 U/L (119). In 2 women, the CK level was slightly elevated (358 U/L and 377 U/L, respectively). The previously reanalyzed muscle biopsies[17] and 1 reanalyzed biopsy in this study all showed nemaline rods, nuclear clumps, and internalized nuclei (Table 1). Seven biopsies also showed cores or core-like areas. The Dutch founder variant in the *KBTBD13* gene (c.1222C>T (p.(Arg408Cys)))[9] was found in 23 patients. One patient with a clinical phenotype of NEM6 had another heterozygous variant at the same locus in the *KBTBD13* gene (c.1222C>A (p.(Arg408Ser))).

Medical History

Only 1 patient reported prematurity, perinatal feeding difficulties, and delayed motor milestones. The symptoms in childhood and the current symptoms are shown in Figure 1. Symptoms since childhood were muscle weakness (n = 23; 96%), slowness of movements (n = 23; 96%), and difficulties with running (n = 20; 83%). The muscle weakness manifested as difficulties in climbing stairs, ring pull-ups, and rope climbing. Running and jumping were slower compared with peers. Muscle stiffness (n = 16; 67% vs n = 10; 42%), myalgia (n = 16; 67% vs n = 4; 17%), and fatigue (n = 15; 63% vs n = 6; 25%) were more prevalent in adulthood than in childhood, respectively.

Most patients experienced limitations in the daily activities in adulthood, including difficulties with getting dressed (n = 18; 75%), performing domestic work (n = 18; 75%), and cycling (n = 21; 88%) (Table 2). Two patients (8%) used a wheelchair for longer distances, which they started to use around the age of 50, and 4 patients (17%) aged between 50 and 59 years used a walking aid. Nine patients (28%) had a cardiomyopathy with a decreased left ventricle ejection fraction based on previous cardiac assessment with echocardiography. Based on Holter monitoring, 4 patients (17%) also had a cardiac arrhythmia (including atrial fibrillation and ventricular tachycardia). Five patients (21%) had an implantable cardioverter/defibrillator. A previous study shows a detailed assessment of these findings.[7] A few patients had respiratory symptoms including recurrent respiratory tract infections (n = 2; 8%) and exertional dyspnea (n = 5; 21%).

Physical Examination

Neurologic examination showed a median MRC sum score of 50 [47.5–54.0] of 60 (Figure 2A). The median MRC scores of individual muscles are shown in Figure 2B; other physical examination results are provided in Table 3. Muscle weakness

Table 2 Patient-Reported Functional Difficulties, Use of Assistive Devices, and Comorbidities

|	Number of patients (%) (n = 24)
Functional difficulties	
Driving a car (n = 21)	13 (62)
Washing	14 (58)
Getting dressed	18 (75)
Domestic work	18 (75)
Cycling	21 (88)
Use of assistive devices	
Wheelchair for longer distances	2 (8)
Walking aid	4 (17)
Comorbidities	
Cardiac	
Cardiac arrhythmia	4 (17)
Decreased left ventricle ejection fraction	9 (38)
Implantable cardioverter/defibrillator	5 (21)
Gastrointestinal	
Constipation	3 (13)
Heartburn	4 (17)
Respiratory	
Recurrent respiratory tract infections	2 (8)
Exertional dyspnea	5 (21)

was symmetrical, and neck flexors were the weakest with a median MRC score of 3. Six patients (25%) showed an abnormal walking gait, consisting of a waddling gait (n = 4) or a mild foot drop (n = 2). Mild muscle atrophy was seen in 12 patients (50%), which was located in the shoulder girdle and/or forearms. Four patients (17%) showed scapular winging, of whom 3 patients showed bilateral winging. Eight patients (33%) showed facial muscle weakness during formal testing (n = 6; 25%) and/or on inspection (i.e., myopathic face) (n = 6; 25%). Most of the patients showed abnormal results on the functional tests; the exception was walking on toes.

Questionnaires on Quality of Life, Fatigue Severity, and Falls

The scores of the SF-36 questionnaire were significantly lower in patients with NEM6 in comparison with the reference group, except for emotional role functioning and emotional well-being concept (Table 4). This reflects a less favorable health state.

The scores of the CIS questionnaire were significantly increased on the perceived fatigue subscale, the concentration subscale, and the total score in comparison with the reference group, reflecting a larger disease burden (Table 4). The mean

Figure 2 MRC Scores, MFM Scores, and Normalized pRR
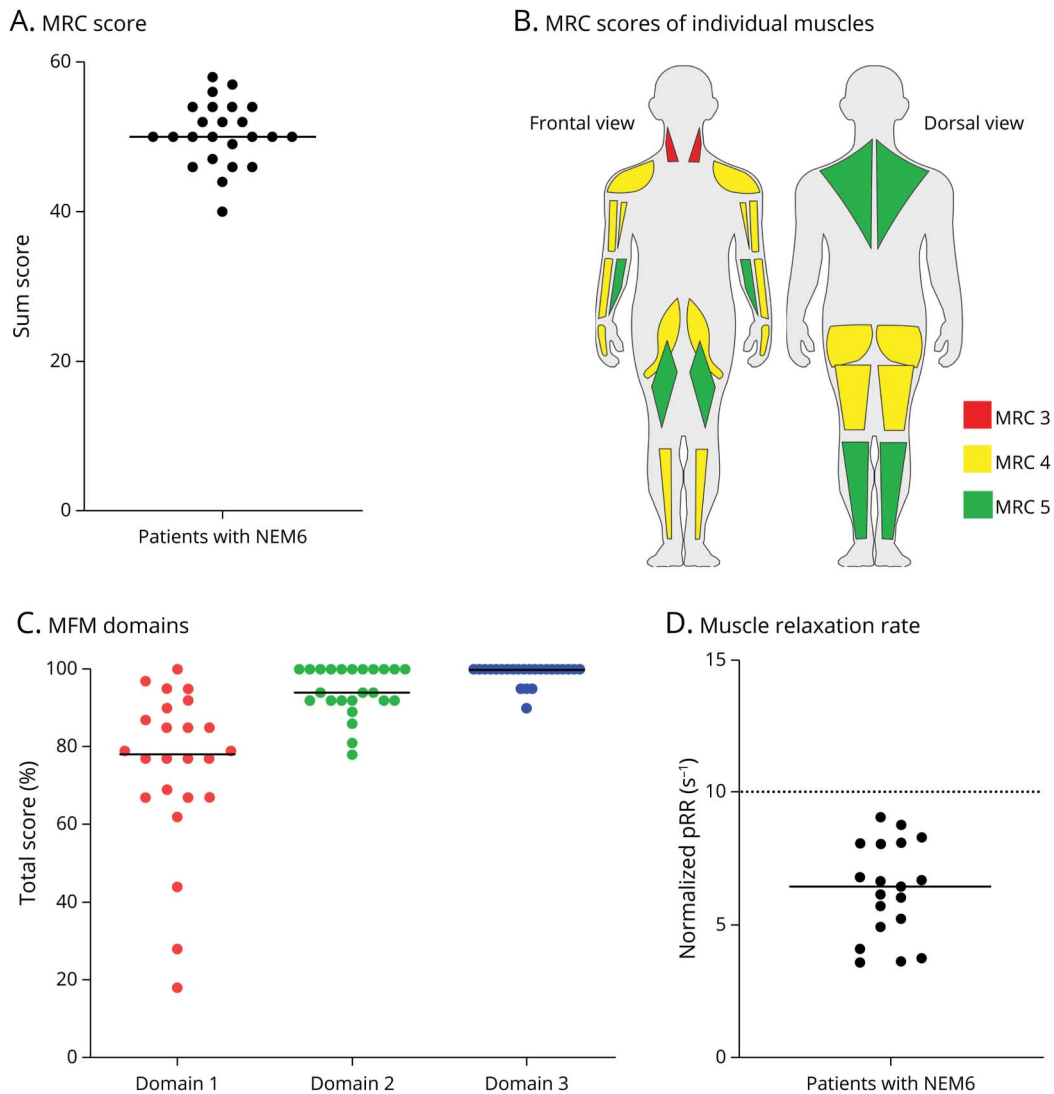
(A) The MRC sum scores of patients with NEM6. The dots represent individual patients and the black horizontal line the median. (B) The median MRC scores of individual muscles with a frontal and dorsal view. (C) MFM scores of the different domains. Domain 1: standing position and transfers. Domain 2: axial and proximal motor function. Domain 3: distal motor function. (D) Normalized pRR in the patients with NEM6. Three patients were not included because of a contraindication or home visits, and 2 patients were excluded because of a silent period <150 ms. The dotted horizontal line represents the lower limit of normal. MFM = Motor Function Measure; MRC = Medical Research Council; NEM6 = nemaline myopathy type 6; pRR = peak relaxation rate.

total score was 77.0 (27.1), and 13 patients (54%) had scores above the cutoff for problematic fatigue (≥76). The mean score on the perceived fatigue subscale was 35.8 (13.6), and 14 patients (58%) reported scores above the cutoff for severe experienced fatigue (≥35).

Seventeen patients (71%) reported falls due to NEM6 in the past. Eight patients (33%) indicated that they fell at least annually, and 9 patients (38%) fell less than once a year. Prospectively, in a period of 100 days, 8 patients (33%) fell at least 1 time, of whom 5 patients (21%) fell twice or more. The reported cause was muscle weakness and slowness of movements and/or muscle stiffness with the subsequent lack of compensation to prevent falling. Patients did not report severe related injuries, such as fractures.

Clinical Tests

The median total score on the Mini-BESTest was 24 [21.0–26.0] and the median total percentage on the MFM was 91% [83.5–95.3], reflecting mild balance and motor impairment, respectively. Patients scored 78% [67.0–89.3] on domain 1, 94% [92.0–100.0] on domain 2, and 100% [100.0–100.0] on domain 3 (Figure 2C). Patients walked a median distance of 551.4 m [518.9–578.5] during the 6MWT. The walk distance of 4 patients (17%) was below the lower limit of normal.

TMS-Induced Muscle Relaxation

Measurement of TMS-induced muscle relaxation rates was performed in 17 patients, of whom 2 patients were excluded because of a silent period <150 ms. Four of the 7 patients who

Table 3 Results of Physical Examination

	Number of patients (%) (n = 24)
Muscle weakness/atrophy	
Scapular winging	4 (17)
Weakness of the facial muscles	6 (25)
Myopathic face	6 (25)
Mild muscle atrophy in extremities	12 (50)
Skeletal abnormalities	
Mild scoliosis	3 (13)
Mild ankle flexion contracture	6 (25)
Lumbar hyperlordosis	7 (29)
High-arched palate	9 (38)
Abnormal functional tests	
Walking on toes	7 (29)
Jumping	21 (88)
Walking on heels	22 (92)
Climbing stairs	22 (92)
Rising from a 30-cm high stool	22 (92)
Running	23 (96)
Rising from squat position	24 (100)
Hopping	24 (100)

The inability to bring the ankle to 0° by passive range of motion was considered an ankle flexion contracture. The functional tests were considered abnormal if the test could not be (fully) performed, if the test was slowly performed, or if there were compensatory movements.

were not able to participate in the TMS experiment because of the presence of an implantable cardioverter-defibrillator or home visits were included in previous studies.[6,39] Hence, the previously acquired relaxation rates of these patients were included in this study. The median NpRR was 6.5 [4.9–8.1] s^{-1} (Figure 2D). All patients showed an NpRR below the lower limit of normal. The median MVC was 213.5 [176.0–243.3] N, and the median percentage of predicted was 66.7% [57.2–84.3]. The MVC of 11 patients (46%) was below the 10th percentile of predicted.

Discussion

The main findings of this comprehensive cross-sectional study in 24 adults with NEM6 are as follows: (1) patient-reported muscle weakness, slowness of movements, and difficulties with running manifest in childhood; (2) proximal, axial, and distal muscles show mild muscle weakness; (3) patients report functional difficulties, which are paralleled by abnormal functional tests; (4) balance control, motor function, and walk distance are mildly affected; (5) experienced health-related

quality of life is decreased and fatigue severity is increased in this patient group compared with a healthy population; (6) CK levels were normal or slightly increased; (7) the presence of self-reported slowness of movements is confirmed by a decreased TMS-induced muscle relaxation rate in all patients and could contribute to a higher prevalence of falls. We discuss the findings as follows.

In adulthood, patients experience functional difficulties in daily life due to muscle symptoms. This contrasts with childhood because patients retrospectively only experienced limitations in performing sports as a child. There is also an increased occurrence of muscle stiffness, myalgia, and fatigue at adult age. These findings could point to a slowly progressive course or reflect that adult patients are expected to perform more complex activities. The reported fatigue is confirmed by the results of the CIS questionnaire. The prevalence of severe experienced fatigue is similar to other neuromuscular diseases, such as facioscapulohumeral dystrophy (61%) and hereditary motor and sensory neuropathy type I (64%).[40]

The distal muscle weakness, in addition to proximal and axial weakness, found in our study is one of the findings that was not reported in the initial NEM6 cohort study.[1] We quantitatively confirmed this finding by a decreased median grip strength, and other studies also reported distal weakness.[3,4] In comparison with the initial NEM6 cohort study, there is also a discrepancy between the MRC scores of the knee extensors. We found a median MRC score of 5 in knee extensors by testing in a standardized way,[18] which is in line with reported strong knee extensors in a previous clinical study.[3] A median MRC score of 3.5 was found in the initial NEM6 cohort study.[1] The most likely explanation is that the latter study tested the knee extensors at higher knee flexion angles because it is known that quadriceps muscle weakness occurs predominantly at higher knee flexion angles in patients with NEM6.[41,42]

A high total MFM score of 91% contradicts the abnormal functional tests. A likely explanation for this high MFM score is the known occurrence of a ceiling effect in mildly affected patients with a neuromuscular disease.[43] Despite this, it is a universally accepted and validated functional clinical outcome measure in patients with a neuromuscular disease. Restriction of use of the MFM to domain 1 would differentiate better in mildly affected patients.

The increased occurrence of falls cannot be explained by an impaired balance control because the Mini-BESTest showed that balance was only mildly affected. Patients report muscle weakness and slowness of movements and/or muscle stiffness with the subsequent lack of compensation to prevent falling as the presumed mechanism for the falls. This is confirmed by a high percentage of patients reporting slowness of movements as one of the symptoms and a decreased TMS-induced muscle relaxation rate in all tested patients. In a previous study performed in patients with myotonic

Table 4 Results of SF-36 and CIS Questionnaires

	Scores of patients with NEM6	Scores of the reference group of healthy population	*p* Value
SF-36 (% of total)			
Physical functioning	57.3 (24.1)[a]	81.9 (23.2)	<0.01
Bodily pain	66.0 (26.2)[a]	79.5 (25.6)	0.02
Physical role functioning	64.1 (31.4)[a]	79.4 (35.5)	0.03
Emotional role functioning	75.0 (31.5)	84.1 (32.3)	0.17
Emotional well-being	70.6 (16.6)	76.8 (18.4)	0.08
Social functioning	70.0 (28.3)[a]	86.9 (20.5)	<0.01
Energy/fatigue	49.4 (23.7)[a]	67.4 (19.9)	<0.01
General health	54.4 (21.3)[a]	72.7 (22.7)	<0.01
Perceived change in health	41.7 (21.7)[a]	52.4 (19.4)	0.02
CIS (score)			
Perceived fatigue	35.8 (13.6)[a]	22.98 (10.75)	<0.01
Concentration	17.6 (7.7)[a]	12.44 (5.96)	<0.01
Motivation	13.5 (6.1)	11.14 (4.74)	0.07
Activity	10.0 (4.7)	8.28 (4.29)	0.08
Total score	77.0 (27.1)[a]	54.84 (21.48)	<0.01

Abbreviation: NEM6 = nemaline myopathy type 6.
The means (SD) of the 36-Item Short Form Health Survey (SF-36) questionnaire are compared with a reference group.[23] A 100% score is the most favorable health state. The means of the Checklist Individual Strength (CIS) are compared with a different reference group.[27] A higher score is indicative of a higher disease burden.
[a] Significant difference in comparison with the reference group.

dystrophy types 1 and 2, the prevalence of falls in 100 days was also prospectively assessed in healthy controls. Less than 10% of these controls fell once and none fell more than once, confirming that the prevalence of falls is increased in patients with NEM6. The mechanism of falling in patients with NEM6 should be further investigated by performing more extensive balance correction studies, for example, by perturbing balance by surface tilt rotations on a platform.[44] Based on our findings, fall prevention strategies taking the slowness of movements into account should be implemented in this group of patients.

One woman had a c.1222C>A (p.(Arg408Ser)) heterozygous variant in the *KBTBD13* gene, which has not been reported as a cause of NEM6 before.[9] Genetic classification resulted in a variant of uncertain clinical significance, based on the high conservation of the encoded amino acid. Moreover, the variant has not been found in a control group of patients in the Genome Aggregation Database and is affecting the same codon as the pathogenic c.1222C>T (p.(Arg408Cys)) *KBTBD13* variant. Based on these findings, this variant is likely to be pathogenic. In addition to the clinical phenotype of the patient, a decreased NpRR of 3.77 s^{-1} and cores and nemaline rods in her muscle biopsy led to the diagnosis of NEM6.

Important findings in our previous studies in patients with NEM6 are cardiac and respiratory manifestations.[8,17] A retrospective study in 65 patients from 3 different families, including most patients in our study, found a high prevalence of cardiomyopathy rendering *KBTBD13* as a novel cardiomyopathy gene. This included 12% with left ventricle dilatation, 29% with a left ventricle ejection fraction <50%, 8% with atrial fibrillation, 9% with ventricular tachycardia, and 20% with repolarization abnormalities.[7] We investigated the presence of respiratory muscle weakness in this cohort in a separate study.[8] Eight of 24 patients with NEM6 showed a forced vital capacity of less than 80% of predicted, indicative of a restrictive lung function and thus respiratory muscle weakness.[45] None of these patients required noninvasive mechanical ventilation. Thus, a referral to the cardiologist and screening of respiratory function are important in all patients with NEM6.

This study has some limitations. First, most patients were (remotely) related, which caused a selection bias. This is inherent to the fact that NEM6 is caused by a Dutch founder variant. Second, another bias is that we included a small number of patients. However, NEM caused by variants in the *KBTBD13* gene are very rare worldwide. This is shown in large genetic cohort studies in patients with a neuromuscular

disease.[46-48] Even in large cohort studies on NEM, patients with NEM6 are sparsely included.[11-15] Thus, owing to this rareness, including large cohorts of patients with NEM6 is very challenging. Third, there was a female predominance, which cannot be fully explained by a selection bias because the previous study also had a female predominance.[1] This reflects the distribution of sex within the identified patients in the Netherlands, making these data more representative for women. Possibly, men are less severely affected, leading to not being diagnosed with NEM6. Because we included only 5 men, the data in this study are insufficient to support this. Fourth, no children older than 6 years were identified, leading to the data only being representative for adult patients. Fifth, if muscle ultrasound or MRI were incorporated in this study, these findings could have been related to disease severity, leading to more knowledge on the pathophysiology. We aim to investigate this in a prospective study. Last, because this is a cross-sectional study, we cannot provide information on the clinical course. A future follow-up study or a future longitudinal study could give more insight into this.

In conclusion, this study contributes to the knowledge on the clinical presentation of patients with NEM6 and gives health care providers a comprehensive clinical overview. Despite the presence of only mild muscle weakness, patients report an important impact on the daily activities, which is illustrated by functional difficulties, reduced quality of life, increased fatigue severity, and increased prevalence of falls. This might be related to the reduced muscle relaxation rate. This overview helps clinicians recognize NEM6, which is important for screening on cardiac and respiratory manifestations. Moreover, this study helps in providing adequate patient information and care.

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Benno Küsters	Department of Pathology, Radboud University Medical Center, Nijmegen, the Netherlands	Drafting/revision of the manuscript for content, including medical writing for content
Jan T. Groothuis	Department of Rehabilitation, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design
Montse Olivé	Neuromuscular Diseases Unit, Department of Neurology, Hospital de La Santa Creu i Sant Pau; Biomedical Research Institute Sant Pau (IIB Sant Pau) Spain; Centro para la Investigación Biomédica en Red en Enfermedades Raras (CIBERER), Barcelona	Drafting/revision of the manuscript for content, including medical writing for content
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Appendix (continued)

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