Neuromuscular Disorders 33 (2023) 580–588

Contents lists available at ScienceDirect

Neuromuscular Disorders

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

Respiratory features of centronuclear myopathy in the Netherlands

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ARTICLE INFO

Article history:
Received 28 December 2022
Revised 6 June 2023
Accepted 9 June 2023

Keywords:
Centronuclear myopathy
Respiratory function
MTM1
BIN1: RYR1
DNM2
Cohort
Netherlands

ABSTRACT

Centronuclear myopathy (CNM) is a heterogeneous group of muscle disorders primarily characterized by muscle weakness and variable degrees of respiratory dysfunction caused by mutations in *MTM1, DNM2, RYR1, TTN* and *BIN1.* X-linked myotubular myopathy has been the focus of recent natural history studies and clinical trials. Data on respiratory function for other genotypes is limited. To better understand the respiratory properties of the CNM spectrum, we performed a retrospective study in a non-selective Dutch CNM cohort. Respiratory dysfunction was defined as an FVC below 70% of predicted and/or a daytime pCO2 higher than 6 kPa. We collected results of other pulmonary function values (FEV1/FVC ratio) and treatment data from the home mechanical ventilation centres. Sixty-one CNM patients were included. Symptoms of respiratory weakness were reported by 15/47 (32%) patients. Thirty-three individuals (54%) with different genotypes except autosomal dominant (AD)-*BIN1*-related CNM showed respiratory dysfunction. Spirometry showed decreased FVC, FEV1 & PEF values in all but two patients. Sixteen patients were using HMV (26%), thirteen of them only during night-time. In conclusion, this study provides insight into the prevalence of respiratory symptoms in four genetic forms of CNM in the Netherlands and offers the basis for future natural history studies.

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1. Introduction

Centronuclear myopathies (CNMs) are a group of inherited neuromuscular disorders which are histopathologically characterized by the presence of nuclei in the centre of muscle cells [1]. CNM is clinically and genetically heterogenous, mainly caused by mutations in *MTM1, DNM2, RYR1, TTN,* and *BIN1* [2–5]. The majority of the corresponding proteins are involved in membrane trafficking and the formation of structures crucial for excitation-contraction coupling, and are thus essential for normal muscle function [6].

CNM is clinically characterized by generalized muscle weakness and hypotonia, often also involving the bulbar and respiratory muscles, leading to nocturnal hypoventilation and/or recurrent chest infections [7]. The age of onset and disease severity is highly variable. In male patients with X-linked *MTM1* mutations (XL-MTM), early death due to respiratory failure is common [8,9]. The clinical outcomes are generally better for other genotypes and

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https://doi.org/10.1016/j.nmd.2023.06.003
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for XL-MTM carriers [10–13]. The high prevalence of respiratory dysfunction in CNM, occasionally disproportionate to the degree of limb girdle weakness, emphasizes the importance of early and regular respiratory screening, even in the absence of suspicious symptoms.

In the past decades, research on respiratory involvement in XL-MTM has been abundant [14–20]. However, data on respiratory function in other CNM genotypes is limited. In a cross-sectional study focusing on the respiratory function of 14 individuals with CNM, thirteen participants required full-time, home invasive mechanical ventilation (HMV), and one only nocturnal non-invasive HMV [21]. Maximal inspiratory pressures (MIP) were higher in subjects who breathed unsupported at least 1 hour/day compared to 24-hour MV users. Furthermore, years of HMV dependence correlated significantly with maximal expiratory pressure (MEP). However, this study included 10 males with XL-MTM, but only one patient with *DNM2*-related CNM, one patient with *RYR1*-related CNM, and two patients without genetic confirmation; age was also highly variable ranging from 1.4 – 27 years. Moreover, there was a selection bias for younger and HMV patients, related to the predominance of XL-MTM patients [21].

Therefore, we performed a nonselective retrospective study focusing on the respiratory characteristics of paediatric and adult CNM patients in the Netherlands. This study was a follow-up of the retrospective study by Reumers et al. on clinical, genetic, and histological features of CNM in the Netherlands [13]. Our main goal was to obtain data on respiratory function, symptoms, and (HMV) of the Dutch CNM cohort. This study is expected to provide insights into the natural history of respiratory function and the use of HMV in the various CNM genotypes prevalent in The Netherlands.

2. Methods

This retrospective, cross-sectional study was performed at the Radboudumc Neuromuscular centre, Nijmegen, in collaboration with the Dutch Neuromuscular centre and the Dutch centres for HMV. Data collection, with regards to historically performed genetic testing (in case of presence of symptoms that indicated a muscle abnormality) and ambulatory status had been collected before by Reumers et al. [13]. This study identified all Dutch patients with CNM referred to our centre between 1998 and 2020. We used the same inclusion criteria and extended the cohort with recently identified patients up to 2022. The study was approved by the local ethics committee (Protocol 2017–4022). All participants, and/or their parents, where applicable, provided written informed consent.

2.1. Patients

Inclusion criteria were 1) a clinical phenotype of congenital myopathy with a (likely) pathogenic variant in one of the main genes associated with CNM: *MTM1, DNM2, RYR1, TTN* and *BIN1*; or 2) a clinical phenotype of congenital myopathy and genetic confirmation in first degree relative(s). Histological confirmation was optional, taking into account the evolving primacy of genetic testing in the current diagnostic setting. Patients were divided into groups per genotype, including a distinction between male patients and female manifesting carriers with an *MTM1* variant.

2.2. Data collection

All CNM patients were identified through four routes [13]: 1) the (paediatric) neurology outpatient clinic at the Radboudumc; 2) the genetics department at the Radboudumc; 3) the Dutch patient association *Spierziekten Nederland*, and 4) (paediatric) neurologists of the Dutch Neuromuscular Centre. Since the Radboudumc is the national referral centre for CNM and is acknowledged as such by the other Dutch neuromuscular centres, this approach was expected to cover a high proportion of Dutch CNM patients.

The data collection item list was prepared in collaboration with the Dutch HMV centers. Clinical patient data were stored in the electronic patient file system of the Radboudumc, and systematically extracted by the researcher (S.B.). Data were pseudonymized and stored in a new phase of the existing Castor database to create an overview of respiratory characteristics of all known CNM patients in the Netherlands. We collected updated medical files from all patients by contacting the four HMV-centres in the Netherlands. In case of missing data, the total number of patients with available data was mentioned. If not specified the total number of patients was 61.

2.3. General clinical features

Data on family history, medical history, clinical features and ancillary investigations were collected in our previous study and were checked again for correctness. The new data extraction sheet used the same set of questions concerning epidemiological and demographic characteristics including year of birth, sex and most recent weight and height. Furthermore, we included items concerning the current status of the patient (alive or deceased), their exact genetic variant, and their age when height and weight were last measured. Presence of scoliosis (and need for surgical scoliosis correction) was assessed. The date of diagnosis was defined as the timepoint when CNM was confirmed, based on genetical and/or histopathological evidence. Ambulatory status was subsequently updated to align with most recent measurements and divided in three separate defined categories: normal, walking with aids, and utilization of a wheelchair.

2.4. Self-reported symptoms of respiratory muscle weakness and bulbar muscle weakness

We collected data of reported symptoms of reduced respiratory muscle strength. We assessed this by the presence of symptoms suggestive of nocturnal hypoventilation (symptoms presented in Table 1). Furthermore, we collected data on reported bulbar muscle weakness, because of the associated risk of aspiration (symptoms presented in Table 1).

2.5. Measured respiratory dysfunction

Respiratory dysfunction was defined as an FVC under 70% of predicted and/or a daytime pCO2 higher than 6 kPa (45 mmHg). Onset of symptoms of respiratory dysfunction was retrieved from medical files. This category entails results from pulmonary function tests (e.g. FVC and FEV1 measurements from spirometry, see Table 2).

We collected both absolute values and values corrected for age, sex, height as the percentage of the predicted value. In the results, we plotted the absolute values in a graph with a line indicating the normal values reported in the literature (Fig. 2) [22,23]. Also, results of arterial or capillary blood gas tests and transcutaneous pCO2 measurements during day and night-time were noted. All pCO2 measurements were transformed to kPa to create a constant value [24]. MIP & MEP were not included since these tests are not part of the standard pulmonary function assessment and many missing data were expected. To check this, we screened for and indeed detected missing MIP & MEP values in the large majority of subjects (>90%). Finally, we collected data on possible pulmonary co-morbidities, such as COPD or OSAS.

Table 1
Overview of specific signs of respiratory dysfunction, nocturnal hypoventilation and bulbar weakness per genotype. At the bottom, three defined categories of ambulatory status were added. Numbers in grey rows indicate the number of patients in whom these data are present, absent or unreported. Numbers in final header shows number of patients with a known ambulatory status. Results presented in numbers of patients and percentage of total patients in that category in brackets .

Respiratory symptoms and signs	AD-BIN1 ($n = 7$)	XL-MTM ($n = 12$)	XL-MTM carriers ($n = 13$)	AR-RYR1 ($n = 10$)	DNM2 ($n = 19$)	Total ($n = 61$)
Symptoms of nocturnal hypoventilation	6/7: absent 1/7 unreported	2/12: present 1/12: absent 9/12: unreported	3/13: present 8/13: absent 2/13: unreported	3/10: present 7/10: absent	7/19: present 10/19: absent 2/19: unreported	15: present
Morning headache	0	0	1 (7.7%)	1 (10%)	3 (15.8%)	5 (15.2%)
Excessive daytime sleepiness	0	1 (8.3%)	0	1 (10%)	3 (15.8%)	5 (15.2%)
Nightmares	0	1 (8.3%)	1 (7.7%)	1 (10%)	1 (5.3%)	4 (12.1%)
Shortness of breath in the morning	0	0	0	0	0	0
Frequent awaking in the night	0	0	0	0	2 (10.5%)	2 (6%)
Feeling exhausted when waking up	0	1 (8.3%)	1 (7.7%)	1 (10%)	6 (31.6%)	9 (27.3%)
Symptoms of bulbar weakness	6/7: absent 1/7: unreported	4/12: present 2/12: absent 6:12: unreported	1/13: present 8/13: absent 4/13: unreported	5/10: present 5/10: absent	8/19: present 9/19: absent 2/19: unreported	19: present
Dysphagia	0	2 (16.7%)	1 (7.7%)	5 (50%)	3 (15.8%)	11 (33.3%)
Dysarthria	0	0	1 (7.7%)	0	3 (15.8%)	4 (12.1%)
Saliva loss	0	2 (16.7%)	0	2 (20%)	0	4 (12.1%)
Open mouth	0	2 (16.7%)	0	2 (20%)	2 (10.5%)	5 (15.2%)
Signs of Resp Dysfunction	0 reported	11 reported	4 reported	5 reported	13 reported	33 reported
FVC < 70% of predicted	0	4 (33.3%)	1 (7.7%)	4 (40%)	7 (36.8%)	16 (48.5%)
Δ FVC sitting and supine > 20%	0	0	0	0	1 (5.3%)	1 (3%)
Daytime pCO2 > 6kPa	0	2 (16.7%)	2 (15.4%)	0	5 (26.3%)	9 (27.3%)
Daytime pCO2 > 45mmHg	0	1 (8.3%)	0	0	1 (5.3%)	2 (6%)
Unknown	0	7 (58.3%)	1 (7.7%)	1 (10%)	1 (5.3%)	10 (30.3%)
Ambulatory status	6 reported	7 reported	10 reported	9 reported	16 reported	48 reported
Normal	3 (50%)	0	7 (70%)	4 (44.4%)	4 (25%)	18 (37.5%)
Walking with aids	3 (50%)	3 (42.9%)	1 (10%)	3 (33.3%)	10 (62.5%)	20 (41.7%)
Wheelchair-dependant	0	4 (57.1%)	2 (20%)	2 (22.2%)	2 (12.5%)	10 (20.8%)

Table 2
Spirometry outcome values per genotype. Columns show either exact values (in case of $n = 1$), ranges (in case of $n = 2$ by a '-' or 3 by a '±') or median [IQR]. Empty boxes mean that there was no data for that category. Pred =% of predicted for age and sex.

	AD-BIN1 ($n = 1$)	Pred-BIN1	XL-MTM ($n = 4$)	Pred-XL-MTM	XL-MTM carriers ($n = 3$)	Pred-XL-MTM carriers	RYR1 ($n = 5$)	Pred-RYR1	DNM2 ($n = 13$)	Pred-DNM2
FVC_sitting (L/s)	6,6 ($n = 1$)	107% ($n = 1$)	1,31 [0,9] ($n = 4$)	32,5% ($n = 2$)	3,59 ± 1,9 ($n = 3$)	80% ($n = 3$)	1,96 [2,9] ($n = 5$)	55,3% ($n = 4$)	2,39 [1,6] ($n = 13$)	56,8% ($n = 12$)
FVC_supine (L/s)	–	–	0,9 ($n = 1$)	–	1,6 - 3,8 ($n = 2$)	80% ($n = 2$)	1,71 + 2,8 ($n = 3$)	54% ($n = 3$)	2,47 [1,6] ($n = 10$)	57,5% ($n = 10$)
FEV1_sitting (L/s)	5,0 ($n = 1$)	98% ($n = 1$)	1,22 [0,7] ($n = 4$)	31% ($n = 2$)	2,20 ± 1,3 ($n = 3$)	71,7% ($n = 3$)	1,82 [2,4] ($n = 5$)	59% ($n = 4$)	2,17 [1,6] ($n = 13$)	57,9% ($n = 12$)
FEV1_supine (L/s)	–	–	0,8 ($n = 1$)	–	1,3 - 2,9 ($n = 2$)	71% ($n = 2$)	1,49 ± 2,1 ($n = 3$)	56,7% ($n = 3$)	1,96 [1,8] ($n = 10$)	58% ($n = 10$)
FEV1/FVC_sitting (%)	75,8 ($n = 1$)	–	85,7 ± 21,7 ($n = 3$)	–	84,1 ± 32,1 ($n = 3$)	–	85 [12,4] ($n = 5$)	–	87 [14,1] ($n = 13$)	–
FEV1/FVC_supine (%)	–	–	–	–	78,1 - 78,4 ($n = 2$)	–	86,5 ± 7,8 ($n = 3$)	–	80,7 [22,7] ($n = 10$)	–
PEF_sitting (L/m)	680 ($n = 1$)	125% ($n = 1$)	115,8–181,2 ($n = 2$)	–	363 ± 84 ($n = 3$)	–	220 [365,8] ($n = 4$)	–	299 [133,4] ($n = 12$)	47% ($n = 1$)
PEF_supine (L/m)	–	–	–	–	319 – 411 ($n = 2$)	–	240 ± 377 ($n = 3$)	–	274 [173,2] ($n = 11$)	32% ($n = 1$)
PCF (L/m)	635 ($n = 1$)	–	175,0–182,4 ($n = 2$)	–	–	–	350 ($n = 1$)	–	150 - 250 ($n = 2$)	–

2.6. Home mechanical ventilation data

We collected treatment data from the HMV centres, including details of home mechanical ventilation, air stacking, use of a cough assist machine, and respiratory strength training.

2.7. Statistical analysis

Data were analysed using IBM SPSS Statistics software (version 25. Armonk, NY: IBM Corp.). Descriptive statistics used were median with interquartile range (IQR) and frequencies with percentages (n(%)). For significance tests, Mann-Whitney *U* test was applied. Because of the retrospective design of this study, missing data were frequent. We have indicated the numbers of different sample sizes in the table and other relevant positions in the results.

3. Results

3.1. Patients

We identified 61 patients with a CNM diagnosis in the Netherlands. Forty-eight of them had been included already in our retrospective clinical study [13], and thirteen additional patients were identified, including five newly diagnosed patients with CNM and eight females with XL-MTM. This final group are female

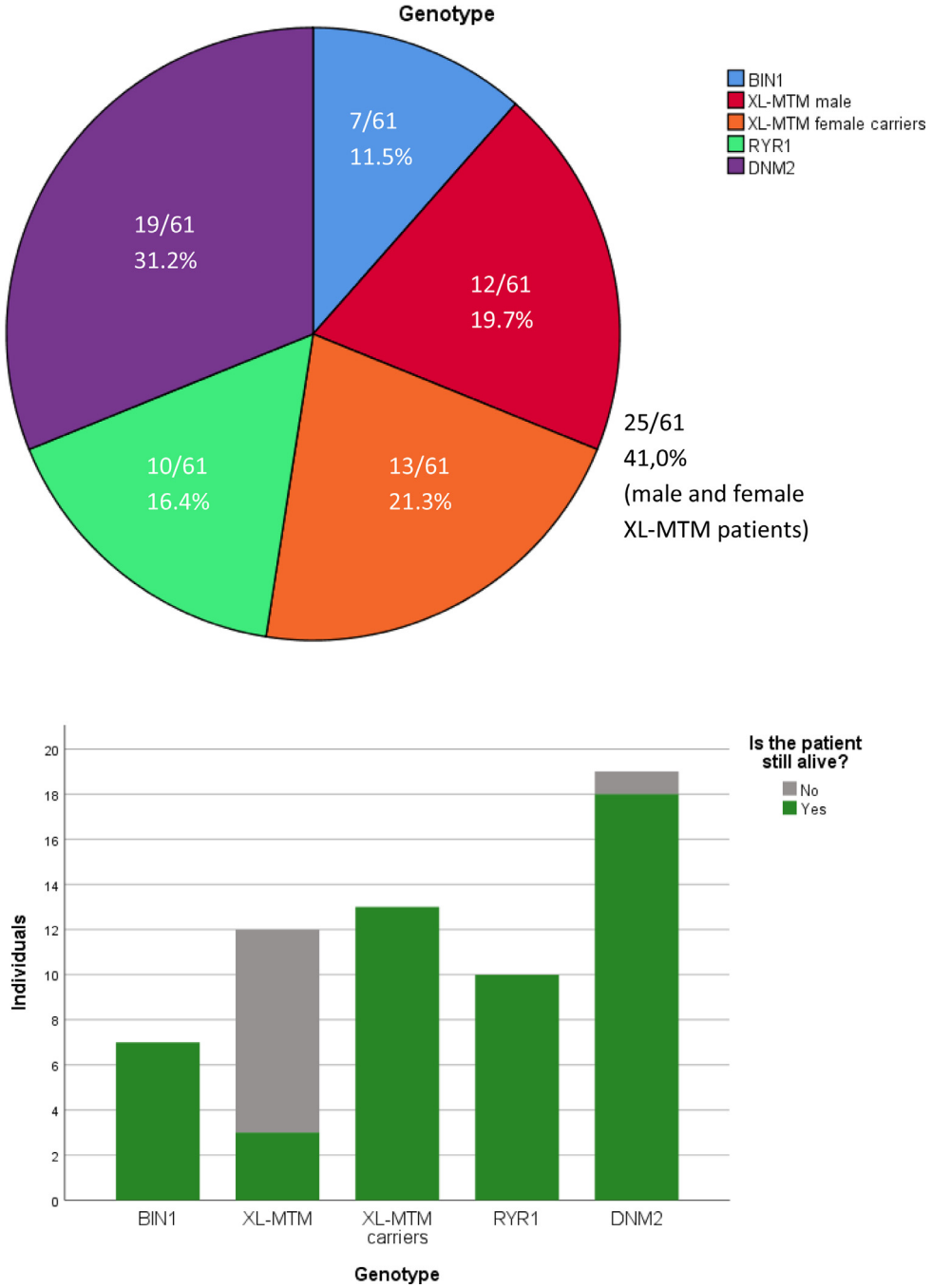

Fig. 1. A: Genotype prevalence in the Dutch CNM cohorts expressed in absolute numbers of the total and percentage. All mutations in *BIN1* were autosomal-dominant, all mutations identified in *RYR1* were autosomal-recessive. B: Number of alive (highlighted in green) and deceased (highlighted in grey) patients per genotype.

carriers and will be addressed as 'XL-MTM carriers. Mean age was 33.5 years, ranging from 0 to 81 years.

The distribution of different genotypes is shown in Fig. 1A and the proportion of alive patients in Fig. 1B. The most common genotype was *MTM1* (n = 25/61, 41%), of which twelve were male. Nine of these twelve males had passed away due to respiratory failure (n = 3/12, 25% survival); seven of those nine died during the first year of life. Five of these nine had been full-time mechanically ventilated until their death. The other prevalent genotypes were *DNM2* (n = 19/61, 31%), autosomal-recessive (AR) *RYR1* (n = 10/61, 16%) and autosomal-dominant (AD) *BIN1* (n = 7/61, 11%). amongst these genotypes, only one other *DNM2* male patient had passed away, unrelated to respiratory dysfunction, at the age of 61. All *BIN1* patients belonged to one family, which has been reported previously [25]. No individuals with *TTN*–CNM were identified.

3.2. Genetic analysis

Genetic confirmation of a known CNM-associated gene had been established in 49 of the 61 participants (80%, criteria 1). The other twelve patients were clinically diagnosed and had a family member with genetic confirmation of CNM (12/61, 20%, criteria 2). This group included the seven XL-MTM boys that died at a very young age, and for whom XL-MTM was genetically confirmed in a first-degree family member. Additional histological confirmation

was achieved in 32 of 61 patients (52%). Results of genetic analysis can be found in our previous study [13] and in Supplementary Table 1.

3.3. Self-reported symptoms of respiratory muscle weakness and bulbar muscle weakness

Symptoms of respiratory muscle weakness were reported by 15 of 47 patients (data not reported for 14 patients, Fig. 2B). This included patients of all genotypes including XL-MTM carriers except AD-*BIN1*. The most common symptom was a feeling of exhaustion upon awakening in the morning (n = 6/19, 32%). Other reported symptoms were frequent awakening at night, morning headaches, and excessive daytime sleepiness.

Bulbar muscle weakness was prevalent in all genotypes except AD-*BIN1* (n = 19/48, 40%). Dysarthria was common in *DNM2* individuals (n = 3/19, 16%) and dysphagia in all *RYR1* patients with respiratory insufficiency (n = 5/10, 50%). No statistically significant differences in sex, age, and other spirometry measurements were found between individuals with or without nocturnal hypoventilation (Supplementary Table 3).

3.4. Measured respiratory dysfunction

Respiratory dysfunction was present in thirty-three patients (n = 33/61, 54%; Fig. 2A). The majority of XL-MTM male (n = 11/12, 92%) and *DNM2* patients (n = 13/19, 68%) showed signs of respiratory dysfunction, while *RYR1* patients (n = 5/10, 50%) and XL-MTM carriers (n = 4/13, 31%) had less severe respiratory impairment. In the case of *DNM2*, a minority showed daytime pCO2 values > 6 kPa (5/19, 26%) (Table 1).

Results of respiratory function tests are summarized in Table 2. Normal values were found in a single patient with AD-*BIN1*. On average, FVC and FEV1 were 32.5 and 31%, respectively, of predicted values in XL-MTM males. In XL-MTM carriers, this value was 80 and 71.7%, respectively. *RYR1* and *DNM2*-mutated individuals showed FVC and FEV1 measurements of 55.3% and 59% for *RYR1* and of 56.8 and 57.9%, respectively, for *DNM2*. The FEV1/FVC ratio, or Tiffeneau index, was between 84 and 87% in all individuals throughout genotypes except those with AD-*BIN1*-related CNM. Supine measurements were overall lower than sitting measurements.

Transcutaneous pCO2 values were available for 18 of our 61 patients (30%) with a mean pCO2 of 5,45 ± 0,66 kPa during daytime (n = 18) and 6,05 ± 0,86 kPa during night-time (n = 15); in eleven cases these numbers were measured before commencement of HMV. Five patients (n = 5/18, 28%) had a daytime kPa value that was above the threshold of 6 kPa (45 mmHg), in line with one of the criteria for respiratory dysfunction.

Next, we compared the values of FVC-sitting, FEV1-sitting, and PEF-sitting with previously reported normal values [22,23] (Fig. 2, for male and female subjects).

3.5. Home mechanical ventilation

DNM2-CNM (n = 11/19, 58%), and *RYR1*-CNM patients (n = 6/10, 60%) were most frequently referred. Seven (4 male, 3 carriers) of the 18 XL-MTM patients who lived longer than 1 year were referred. None of the AD *BIN1* was referred to an HMV-centre. In total, 24 patients received an invitation for an appointment at an HMV centre after assessment in a hospital. The mean age of referral was 31 ± 20 years (range 1 – 63). The mean age at which HMV treatment was initiated was 29 ± 21 years (range 1–65). This difference is explained by the fact that the eight patients that did not need to start HMV were older. We also identified a 62-year-old man who was referred to an HMV centre at 61 but declined because he did not want to start HMV.

Of those 24 patients, 16 initiated HMV (67% of 24 referred and 26% of the total of 61) (Fig. 3). Seven male XL-MTM patients received continuous respiratory support at the hospital from birth until death in the neonatal period and two alive patients do not receive HMV, which makes XL-MTM the most prevalent genotype on ventilation. Fig. 4 shows the distribution amongst the various genotypes and the number of off-ventilation hours per day. Three XL-MTM male patients received mechanical ventilation at home in the past, currently this number is down to one. Other prevalent genotypes on ventilation were *DNM2* with eight patients (n = 8/19, 42%), followed by *RYR1* (n = 3/10, 30%).

In total, only three patients (XL-MTM, male 11 years , AR *RYR1*-CNM, male 9 years and AD *DNM2*-CNM, female 13 years) received daytime and night-time ventilatory support at home, two of whom could breathe independently for a maximum of two hours. The other 13 patients have been diagnosed with nocturnal hypercapnia and only needed nocturnal HMV. Their off-ventilator times differ from 13 h to 17 h per day.

Fourteen patients (14/16, 88%) received some form of additional supportive therapy to improve their respiratory function: 11 were advised to perform air stacking, and two used a cough assist machine. One patient had started singing as a functional remedy to improve respiratory function.

3.6. General clinical features

Scoliosis (n = 6/61, 10%) was observed in *MTM1* (n = 3) and *DNM2* patients (n = 3), of whom one patient had undergone surgical correction at the age of 18. Furthermore, 4 patients (7%) needed a gastrostomy tube.

In our cohort, 20 patients showed at least one pulmonary co-morbidity (Supplementary Table 2). Most frequent were pneumonia (n = 7/20, 35%), pneumothorax (n = 5/20, 25%) and COPD/OSAS (both n = 3/20, 15%).

Our cohort included 6 smokers (30%) and 10 participants that consumed alcohol regularly (50%, mean = 1,3 units per day). Finally, five patients (25%) have been admitted for a respiratory tract infection in the preceding five years, often associated with pneumonia or pneumothorax. One female XL-MTM-carrier was admitted five times with a total of six episodes of pneumothorax, while another DNM2-CNM patient was admitted three times with four episodes of pneumonia. 4 of the 5 pneumothorax patients were diagnosed for XL-MTM (3 female and 1 male). The final individual is the same DNM2-male that passed away due to reasons unrelated to respiratory dysfunction as mentioned in Section 3.1.

3.7. Ambulation and respiratory dysfunction

Ambulatory status was available for 48 of the 61 patients: 18 (38%) had normal ambulation, 20 (42%) were ambulant with aids, and 10 (5%) were utilizing a wheelchair (Table 1). Normal ambulation was present in 4 of 16 (25%) *DNM2* patients, none of the male XL-MTM patients, 7 of 10 (70%) XL-MTM carriers, 4 of 9 (44%) *RYR1* and 3 of 6 (50%) AD *BIN1* patients. An overview of genotype combined with ambulatory status is depicted in Fig. 1B. Out of 38 individuals that had either normal ambulation or were ambulant with aids, 18 showed signs of respiratory dysfunction (47%). However, only 10 (26%) patients received HMV, six of whom were significantly impaired with walking and four of whom were mildly impaired requiring walking aids. The individuals with normal ambulation had no signs of respiratory dysfunction.

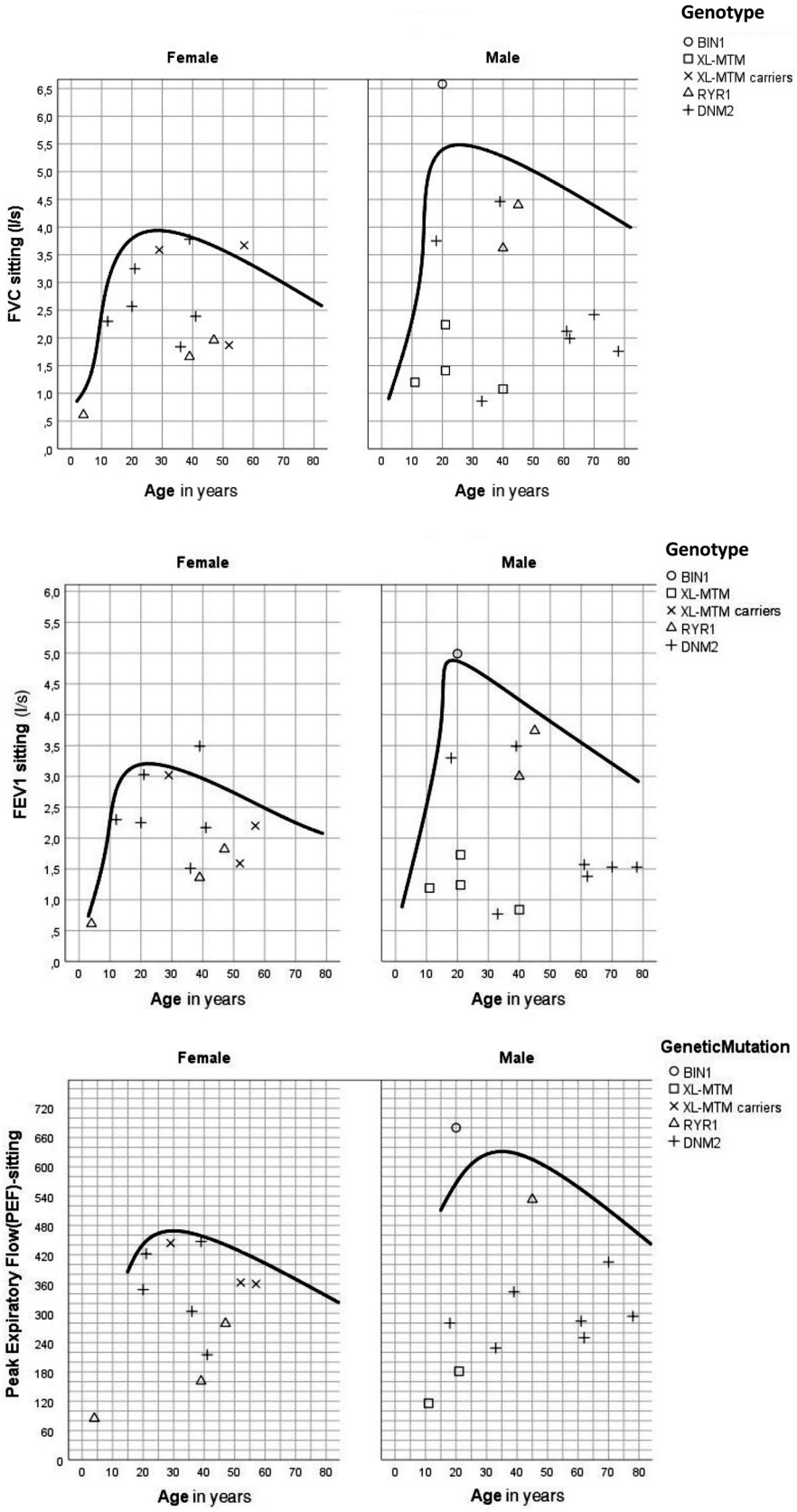

Fig. 2. A-C: Diagram of FVC, FEV1 and PEF-measurements found in Dutch individuals distributed by sex and genotype (visualized by shape). The black line shows predicted normal values at different ages. Genotypes are indicated at the top right of each graph.

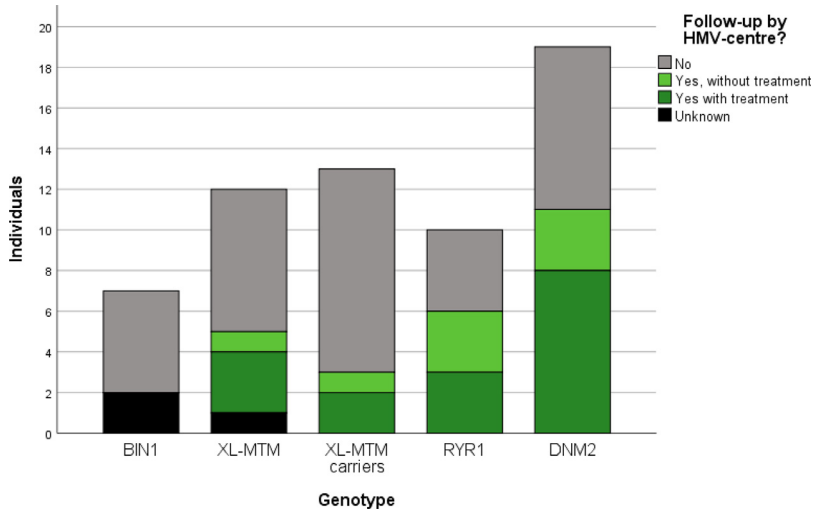

Fig. 3. Number of patients per genotype that were referred to an HMV-centre (category 'Yes') and number that initiated home mechanical ventilation (category 'Yes, with treatment').

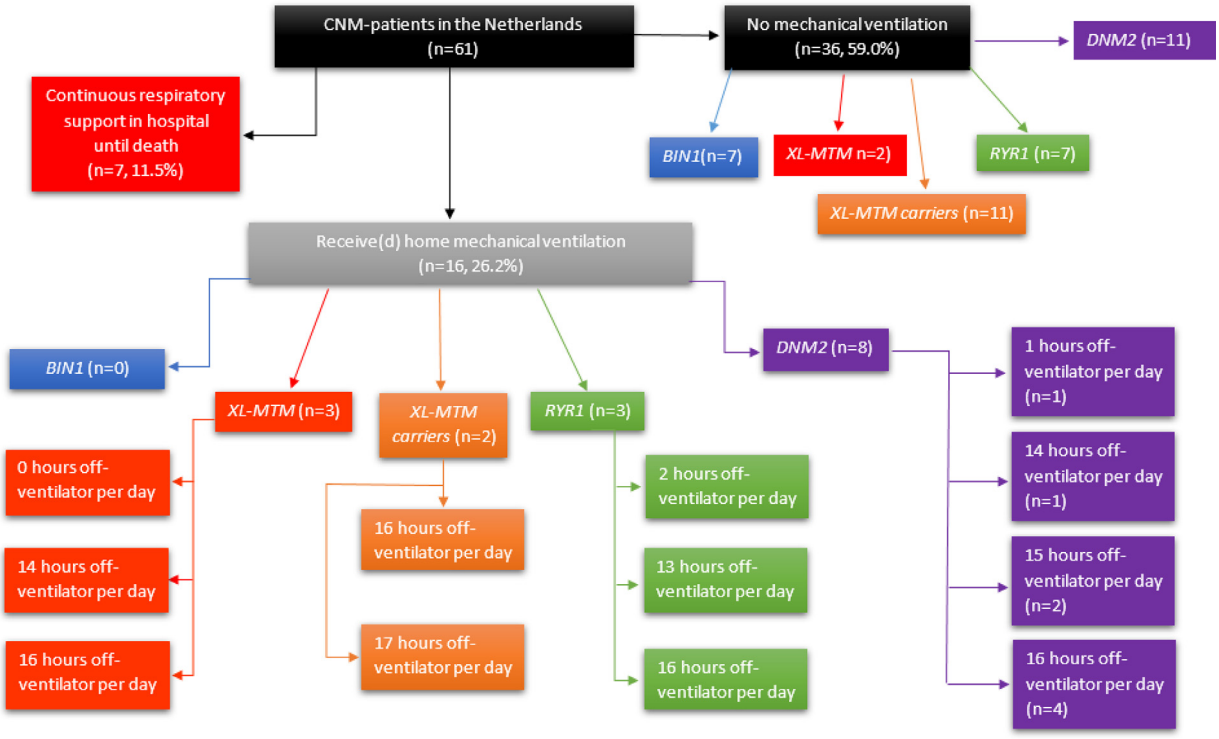

Fig. 4. Visualization of all CNM-patients in the Netherlands, their ventilation status and (where applicable in ventilated patients) the number of hours off-ventilator. Colour of the box corresponds to genotype of the patients in that category.

4. Discussion

This nationwide retrospective study comprehensively summarizes respiratory signs and symptoms of the Dutch CNM cohort. Respiratory dysfunction was found in all genotypes except AD *BIN1*. In line with previous studies, most XL-MTM males were severely affected, resulting in frequent premature death (75%) after a period of ventilatory support. Symptoms of respiratory muscle weakness (nocturnal hypoventilation) were also prominent in AD *DNM2*- and AR *RYR1*-related CNM. Pulmonary function studies showed that FVC, FEV1, and PEF are lower in almost all CNM patients with a *MTM1, DMN2*, or *RYR1* variant. Finally, we found sixteen cases who (had) received home mechanical ventilation, distributed throughout all genotypes except AD-*BIN1*. These results illustrate respiratory features of CNM in the Netherlands in a non-selective way, and provide preliminary insights into the natural history of respiratory involvement across different genotypes and throughout the lifespan.

This study is a follow-up to an earlier study, which was the first to report the total cohort of CNM patients in the Netherlands [13]. This initial cohort of 48 was expanded to 61 cases, reflecting the inclusion of newly diagnosed XL-MTM carriers and other CNM patients since the publication of the initial study. *DNM2*-related CNM was the most common genotype. This corresponds to previously published findings in Italy and Denmark [26,27]. In other studies, AR *RYR1*-related CNM was a more common genotype [28,29].

The large proportion of *DNM2*- and AD *BIN1*-related CNM in this cohort limits the direct extrapolations of findings to other countries. The high prevalence of *DNM2*-related CNM contradicts a recent epidemiological model [30], suggesting that *MTM1* is the most common CNM-associated genotype. There are several reasons for this discrepancy. First, prenatal genetic testing is widely available in the Netherlands, and abortion is allowed until at least 24 weeks in case of an affected foetus [13,31]. Moreover, the Netherlands has a long history to electively discontinue continuous respiratory support in infants without any prospect of functional improvement, in contrast to the USA [32] or Japan. This practice influences death rates and likely skews the functional outcomes to those with higher level of function (i.e. the stronger phenotypes). In general, the access to and decision-making around the range of respiratory support skews the "natural" outcomes in many rare diseases, and in an age of increasing interventions there is no such thing as a "natural" history study any longer. Finally, our centre has both a paediatric and adult neuromuscular service, therefore including patients of all ages and severities. We therefore propose that the international variation in access to and decision-making around the range of respiratory support should be taken into account in the design of future prospective studies. Epidemiological expertise can be used to correct for different practices of prenatal screening and postnatal treatments in different populations.

Another remarkable finding was the large variability within the specific genotypes, which might be as or even more relevant than the differences between the different genotypes. The implication for future studies would be that scientists should not value group medians or normal values too much. Run-in periods before start of the study drug are very important since it enables to assess the development of respiratory function over time in individual trial participants. This can take place as standardized assessment in clinical care or as natural history study preceding the trial. Bayesian statistical analysis of these natural history data enable a simulation of the evolution of symptoms for individual patients over time and to probabilistically compare these simulated trajectories to measured post-treatment outcomes. Further research and ongoing dialogue with regulatory authorities are needed to allow for this and other applications of Bayesian statistics in orphan disease research [33].

This study included a number of other important clinical observations. First, it confirmed that XL-MTM in males is the most severe variant of CNM [9]. The cohort included nine males with XL-MTM, five of whom died within weeks after start of continuous ventilatory support, either due to complications ($n = 2$) or following withdrawal of ventilation ($n = 3$). Two survived longer but not beyond the age of one year. Two other male XL-MTM patients died at the age of 11 and 40 years, respectively, due to progressive respiratory weakness. The only other patient that had passed away was a male *DNM2* patient, who died of an unrelated cause at the age of 61 He did not receive ventilatory support at the time of death. Next, pneumothorax has only sporadically been reported in CNM [34–36], however recently three carriers were described one of whom reported recurrent episodes , one of whom also included in this study. Finally, respiratory dysfunction was absent in AD *BIN1*-related CNM, in contrast with AR *BIN1*-related CNM [37].

Next, half of the AR *RYR1*-related CNM patients ($n = 5$) showed signs of bulbar weakness, with dysphagia present in all of them. Bulbar weakness was also a common feature in patients with *RYR1* mutations [5]. This was more frequent than previously reported [38,39] and has important implications for rehabilitation. In neuromuscular disorders, bulbar muscle weakness often results in pharyngeal residue after swallow [40]. Post-swallow residue is assumed to pose a risk for aspiration on subsequent swallows [41], and may subsequently result in reduced respiratory function [42]. Therefore, speech therapist and dieticians should provide optimal symptomatic management. Symptoms of bulbar weakness has also been described in research analysing the effects on the quality of life (QoL) in patients with spinal bulbar muscular atrophy. They also found problems with swallowing and difficulty talking and identified numerous symptoms that affected QoL, including previously unknown symptoms. Similar research for patients with CNM could benefit clinical care for patients and improve potential future clinical trials [43].

Furthermore, the assessment of respiratory function based on spirometry and CO2 in arterial blood gas or transcutaneous pCO2 monitoring was hampered by different protocols used and many missing data. Supine measurements were frequently missing, in some patients because sitting FVC, FEV1 & PEF measures were sufficiently impaired to indicate the need for respiratory support. In addition, some patients were physically unable to perform supine tests. Furthermore, we included PCF measurements as a test to assess cough in patients with respiratory muscle weakness [44], however, these data were only available in six patients. Next, the analysis of the predicted FEV1/FVC ratio often resulted in ratios close to or even higher than 100%, up to a maximum of 115%. This reflects the respiratory impairment in absence of obstructive lung disease. To overcome missing data for normalized outcomes, we used the absolute data of our three most common parameters, FVC, FEV1, and PEF and compared the results with previously reported normal values for age and sex (Fig. 2). Finally, pCO2-values were only available in 18 of the 61 patients. These tests were likely performed less frequently because spirometry supplied sufficient information. These observations call for standardisation in clinical assessments of respiratory function of patients with CNM and other congenital myopathies, which will be of importance for quality of care and possibly also for the collection of run-in data for future trials.

The data collection of referrals to HMV centers provided additional interesting insights: Twenty-four patients were assessed at an HMV-centre (39%), and 16 of them initiated HMV after this referral (26%). We found a indicative link between ambulatory status and respiratory dysfunction: only four ambulant (with our without support) individuals received HMV (11%), whereas six wheelchair-dependant individuals were on HMV (60%) [19].

This study has a number of strengths and limitations. First, the retrospective design enabled us to include all patients known at our centre, thus reducing selection bias and inclusion of a wide range of outcome measures. |Other strengths are the combination of data on respiratory and bulbar weakness, together with spirometry values and ambulation status, as well as the close collaboration with nationwide HMV centers. Based on our findings, centers of expertise can start or continue collecting data on respiratory function systematically to provide outcomes for future studies. However, the study design led to many missing data, including MIP/MEP values; we had anticipated this and therefore not included these measurements in the protocol. Furthermore, the medical history was retrieved from notes in medical files obtained during history taking and not systematically assessed. This is also likely to result in incomplete data. Other limitations are the fact that this was only a Dutch cohort; that the cohort did not include all genotypes (e.g. not AR *BIN1* patients); and that there was a large variation in measures used in the different Dutch centres.

In conclusion, this retrospective study showed that respiratory characteristics in CNM vary greatly within and between genotypes and individuals in the Dutch cohort. The standardisation of respiratory testing should be promoted in clinical care and research. This is of great importance in the light of emerging therapies. Future natural history studies and innovative statistical methods may help to improve the generalisability of results.

Declaration of Competing Interest

N. Voermans was PI of the phase I/II Unite-CNM trial for Dynacure. This has not caused a conflict of interest for this manuscript. None of the other authors reported a conflict of interest. The authors received no financial support for the research, authorship, and/or publication of this article.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

We would like to thank all neuromuscular neurologists and clinical geneticists who contributed patients to our study cohort. Furthermore, we thank Spierziekten Nederland for the dissemination of information about this study. We also thank Belinda Cowling from Dynacure inc. for contributing to the discussion on respiratory function in CNM. Several authors of this paper are members of the Netherlands Neuromuscular centre (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.2023.06.003.

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