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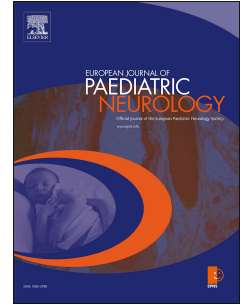
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1 **Respiratory function in LAMA2-related muscular dystrophy and SELENON-related congenital**  
2 **myopathy, a 1.5-year natural history study**

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23

**1 Abbreviations**

- 2 ACMG = American College of Medical Genetics and Genomics
- 3 BMI = body mass index
- 4 DTend-exp = diaphragm thickness end expiratory
- 5 DTmax-insp = diaphragm thickness maximal inspiratory
- 6 DTR = diaphragm thickness ratio
- 7 dVC% = percentage decrease in vital capacity from the upright to the supine position
- 8 ECMO = extracorporeal membrane oxygenations
- 9 FEV1% = percentage predicted of forced expiratory volume in the first second
- 10 FVC% = percentage predicted of forced vital capacity
- 11 IQR = interquartile range
- 12 ITend-exp = intercostal muscle thickness end expiratory
- 13 LAMA2-MD = LAMA2-related muscular dystrophy
- 14 MEP = maximal expiratory pressure
- 15 MFM-20/32 = motor function measurement 20/32
- 16 MIP = maximal inspiratory pressure
- 17 PCF = peak cough flow
- 18 SELENON-RM = SELENON-related congenital myopathy
- 19 SNIP = sniff nasal inspiratory pressure
- 20 VC = vital capacity
- 21

**1 Abstract**

2 INTRODUCTION: LAMA2-related muscular dystrophy (LAMA2-MD) and SELENON(SEPN1)-related  
3 congenital myopathy (SELENON-RM) are rare neuromuscular diseases with respiratory impairment from a  
4 young age. Prospective natural history studies are needed for prevalence estimations, respiratory  
5 characterization, optimizing clinical care and selecting outcome measures for trial readiness.

6 METHODS: Our prospective 1.5-year natural history study included spirometry (forced vital capacity (FVC);  
7 difference between upright and supine vital capacity (dVC)), respiratory muscle strength tests (sniff nasal  
8 inspiratory pressure (SNIP)) (age $\geq$ 5 years), and diaphragm ultrasound (thickness; thickening; echogenicity; all  
9 ages).

10 RESULTS: Twenty-six LAMA2-MD patients (M=8, median 21 [9; 31] years) and 11 SELENON-RM patients  
11 (M=8, 20 [10; 33] years) were included. At baseline, 17 (85%) LAMA2-MD (FVC%: 59% [33; 68]) and all  
12 SELENON-RM patients (FVC%: 34% [31; 46]) had an impaired respiratory function (FVC% $<$ 80%). Nine  
13 (35%) LAMA2-MD and eight (73%) SELENON-RM patients received mechanical ventilation at baseline, and  
14 two additional SELENON-RM patients started during follow-up. Contrarily to LAMA2-MD, SELENON-RM  
15 patients had severe diaphragm atrophy (diaphragm thickness z-score: -2.5 [-3.1; -2.1]) and dysfunction  
16 (diaphragm thickness ratio: 1.2 [1.0; 1.7]; dVC: 30% [7.7; 41]). SNIP was low in both neuromuscular diseases  
17 and correlated with motor function. In SELENON-RM, respiratory function decreased during follow-up.

18 CONCLUSION: The majority of LAMA2-MD and all SELENON-RM patients had respiratory impairment.  
19 SELENON-RM patients showed lower respiratory function which was progressive, more prevalent mechanical  
20 ventilation, and more severe diaphragm atrophy and dysfunction than LAMA2-MD patients. Spirometry  
21 (FVC%, dVC) and respiratory muscle strength tests (SNIP) are useful in clinical care and as outcome measure  
22 in clinical trials.

23

24 Clinical trial number: NCT04478981

25

26 **Keywords:** LAMA2-related muscular dystrophy; SELENON-related congenital myopathy; mechanical  
27 ventilation; respiratory function; respiratory muscle strength; diaphragm

28

## 1 1. Introduction

2 LAMA2-related muscular dystrophy (LAMA2-MD) and SELENON(SEPN1)-related congenital myopathy  
3 (SELENON-RM) are rare neuromuscular diseases with remarkable similarities in clinical phenotype. They are  
4 characterized by slowly progressive axial and proximal muscle weakness, respiratory impairment, early-onset  
5 spinal rigidity, scoliosis and low bone quality<sup>(1-5)</sup> (Bouman et al. 2023, in press). LAMA2-MD and SELENON-  
6 RM are caused by pathologic variants in the *LAMA2* and *SELENON (SEPN1)* genes, coding for the laminin  
7 alpha2 subunit and selenoprotein N, respectively<sup>(6,7)</sup>. No curative therapies exist but promising preclinical trials  
8 are ongoing. These expected trials emphasize the need to reach trial readiness<sup>(8-13)</sup>. In order to optimize physical  
9 condition and to prevent or treat severe complications, patients receive rehabilitation, respiratory care,  
10 orthopedic management and nutritional guidance<sup>(14,15)</sup>.

11 Life expectancy is not well documented, but death as early as the first decade has been described in LAMA2-  
12 MD and SELENON-RM patients that are severely affected<sup>(1,2,16)</sup>. Respiratory impairment is both in LAMA2-  
13 MD and SELENON-RM the leading cause of morbidity and mortality, which makes it a topic of high  
14 importance for clinical care and an essential outcome measure for future clinical trials<sup>(3)</sup>. In LAMA2-MD  
15 patients, non-invasive mechanical ventilation is mostly needed in severely affected patients. Respiratory  
16 impairment follows a restrictive pattern similar to most other neuromuscular diseases with respiratory  
17 involvement<sup>(16)</sup>. Intercostal muscle weakness, decreased compliance of the chest wall and thoracic deformities  
18 related to scoliosis are mainly recognized as the underlying cause of respiratory impairment in LAMA2-MD<sup>(3)</sup>.  
19 Despite that the diaphragm is the most important respiratory muscle, its function has not been well-documented  
20 in LAMA2-MD, although it has been hypothesized that diaphragm function is relatively spared<sup>(3,17)</sup>. In  
21 SELENON-RM, respiratory involvement is strikingly disproportionate to limb weakness: most patients require  
22 mechanical ventilation, mainly nocturnal non-invasive mechanical ventilation, while still being ambulant<sup>(1)</sup>.  
23 Weakness of inspiratory and expiratory muscles, scoliosis and thoracic deformities contribute to respiratory  
24 failure<sup>(1)</sup>. Additionally, based on transdiaphragmatic pressure measurements it has been hypothesized that  
25 diaphragmic weakness plays an important role in the underlying pathophysiology of respiratory impairment<sup>(18)</sup>.

26 In both LAMA2-MD and SELENON-RM, prevalence estimations on respiratory impairment and mechanical  
27 ventilation, and respiratory characterization are lacking. In SELENON-RM, prospective natural history data are  
28 also absent. Further, diaphragm ultrasound, which is increasingly used in the diagnosis of diaphragm  
29 dysfunction, combined with diaphragm function assessments are missing<sup>(19,20)</sup>. The role of the diaphragm in the  
30 pathophysiology of respiratory impairment in LAMA2-MD and SELENON-RM is thus not well understood.

1 Moreover, apart from general congenital muscular dystrophy and congenital myopathy guidelines or guidelines  
2 based on expert opinion, no disease-specific recommendations on respiratory care and on the selection of  
3 outcome measures for clinical trials in LAMA2-MD and SELENON-RM exist<sup>(14, 15, 21)</sup>. Finally, correlations  
4 between respiratory function, respiratory muscle strength, age and motor function are underreported in the  
5 literature. Here we present a prospective 1.5-year natural history study on respiratory involvement in patients  
6 with LAMA2-MD and SELENON-RM that aims to fill in these knowledge gaps.

7 LAMA2-MD and SELENON-RM are jointly discussed in this manuscript since they are both ultrarare and have  
8 remarkable similarities in clinical phenotype. Consequently, LAMA2-MD and SELENON-RM fit in the same  
9 natural history study protocol and we aimed to use the efficiency of a *basket natural history study*<sup>(22)</sup>. We  
10 expected to learn from differences between these neuromuscular diseases. Moreover, promising new therapies  
11 are being developed and there is a high need for trial readiness in both neuromuscular diseases.

## 13 2. Methods

### 14 2.1 Study design and population

15 Patients were recruited non-selectively and consecutively in the period from August 2020 to May 2022 as part  
16 of the LAST STRONG Study, a 1.5-year prospective natural history study in patients with LAMA2-MD or  
17 SELENON-RM. An elaborate description of the protocol can be found elsewhere<sup>(23)</sup>. We aimed to reach all  
18 Dutch-speaking patients in the Netherlands and Flanders. All patients and/or their legal representatives provided  
19 informed consent prior to their inclusion in the study and procedures were performed in accordance with the  
20 ethical standards laid down in the declaration of Helsinki. Inclusion criteria were a genetic confirmation of  
21 LAMA2-MD or SELENON-RM by two recessive (likely) pathologic variants in the *LAMA2* or *SELENON*  
22 (*SEPN1*) gene following the American College of Medical Genetics and Genomics (ACMG) guidelines, or  
23 typical clinical and histological alterations combined with genetic confirmation in a first degree relative<sup>(24)</sup>.  
24 Exclusion criteria were an insufficient understanding of the Dutch language.

25 All patients were invited for four visits within 1.5 year (every six months). The protocol included a standardized  
26 medical history examination, spirometry, respiratory muscle strength tests and ultrasound of the diaphragm and  
27 intercostal muscles. Further, patients underwent an X-ray of the spine at baseline and after one year follow-up.  
28 In case patients did not wish or were not able to visit our hospital, they were offered to participate through home  
29 visits with only medical history examination and respiratory muscle strength tests.

30

## 1 2.2 Clinical features

2 Demographic data were systematically collected from all patients. Motor function was assessed through the  
3 motor function measurement 20/32 (MFM-20/32). Ambulation was defined as being able to walk 10 meters  
4 without support. Rigid spine was defined by a decreased flexibility in the neck or lower back. Patients were  
5 asked for respiratory symptoms and the use of mechanical ventilation (none, nocturnal non-invasive, nocturnal  
6 and daytime non-invasive or invasive mechanical ventilation).

## 8 2.3 Spirometry and respiratory muscle strength tests

9 All patients (age  $\geq 5$  years) that visited our hospital performed spirometry with a handheld spirometer  
10 (SpiroUSB, Vyair Medical connected to PC Spirometry software, Spida CareFusion 2.3.0.10 for Windows 7).  
11 Forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) in sitting position were  
12 measured, compared with reference values and expressed as percentage predicted (FVC% and FEV%,  
13 respectively)<sup>(25)</sup>. Restrictive pulmonary function was defined as FVC $<80\%$ <sup>(26)</sup>. Peak cough flow (PCF) was  
14 assessed in the sitting position and was considered abnormal if  $<270$  l/min<sup>(27)</sup>. Further, vital capacity (VC) was  
15 assessed both in the sitting and supine position, and percentage decrease in VC between the sitting and supine  
16 position was calculated according to the following equation:  $dVC\% = (VC_{\text{upright}} - VC_{\text{supine}})/VC_{\text{upright}}$  and  
17 was considered abnormal if  $>10\%$ <sup>(17)</sup>. Respiratory muscle strength tests included maximum inspiratory pressure  
18 (MIP), maximum expiratory pressure (MEP) and sniff nasal inspiratory pressure (SNIP). MIP, MEP and SNIP  
19 were performed in the upright position with a handheld electronic manometer (Micro RPM, Micro Medical,  
20 CareFusion, United Kingdom) in all patients (age  $\geq 5$  years), and were expressed as absolute values. Both  
21 spirometry and respiratory muscle strength tests were performed in accordance with the American Thoracic  
22 Society/European Respiratory Society (ATS/ERS) statement on respiratory muscle testing<sup>(19, 20, 28)</sup>. We used a  
23 breathing mask instead of a standard mouth piece in case of macroglossia, insufficient closure of the mouth or in  
24 the presence of any other factors that might cause air leakage during spirometry or respiratory muscle strength  
25 tests.

## 27 2.4 Ultrasound of the diaphragm and intercostal muscles

28 Ultrasound of the diaphragm was performed in the supine position in all visits using an Esaote MyLab Twice  
29 ultrasound machine (Esaote SpA, Genoa, Italy) equipped with a 3-13 MHz LA533 linear transducer (Esaote  
30 SpA, Genoa, Italy) according to previously described methodology<sup>(29)</sup>. In short, diaphragm thickness and



1 thickening were assessed bilaterally in the supine position. The ultrasound probe was placed at the zone of  
2 apposition of the diaphragm, typically at the antero-axillary line in the 8<sup>th</sup> or 9<sup>th</sup> intercostal space. Thickness was  
3 measured at resting end-expiration (DTend-exp), and at maximal end-inspiration (DTmax-insp), from the  
4 superficial part of the peritoneal layer to the deep part of the pleural layer. A non-identifiable diaphragm was  
5 defined as a thickness of < 0.1 mm. Standardized scores (z-scores) of DTend-exp were calculated as the number  
6 of standard deviations from the predicted value and were considered abnormal if < -2. Diaphragm thickening  
7 ratio (DTR) was calculated as DTmax-insp/DTend-exp and was considered abnormal if < 1.6<sup>(29)</sup>.

8 Echogenicity of the diaphragm was measured at end-expiration by calculating the mean grayscale level within a  
9 manually selected region of interest in three ultrasound images using an in-house developed software package in  
10 MATLAB (version 2013b, Mathworks, Natick, MA, USA)<sup>(23)</sup>. Standardized scores (z-scores) were calculated as  
11 the number of standard deviations from the predicted value and were considered abnormal if > 2. In case the  
12 DTend-exp was less than 1 mm, echogenicity of the diaphragm could not be reliably measured and was thus  
13 excluded for further analysis.

14 Thickness of the parasternal intercostal muscles was measured 2-3 cm lateral to the sternum in the second or  
15 third intercostal space at resting end-expiration (ITend-exp) from the superficial part of the pleural layer to the  
16 deep part of the pectoralis major fascia at all visits.

### 17 18 *2.5 X-ray of the spine*

19 The presence of scoliosis was assessed by X-ray of the spine at baseline and after 12 months, at the 3<sup>rd</sup> visit, and  
20 was dichotomized as present (Cobb Angle  $\geq 10$  degrees) or absent. The X-ray of the spine was performed in the  
21 sitting position, or in the lying position in case a patient was not able to maintain the sitting position, and the  
22 position was held constant between two consecutive X-rays. Scoliosis was subsequently subdivided into mild  
23 (Cobb's angle 10-20 degrees), moderate (Cobb's angle 21-40 degrees) and severe (Cobb's angle > 40 degrees),  
24 and was classified independent of the presence of scoliosis surgery material<sup>(30)</sup>. In case only one X-ray was  
25 performed (i.e. death, loss from follow-up, practical difficulties), the Cobb's angle measured on this X-ray was  
26 used throughout the study.

### 27 28 *2.6 Data analysis and statistical methods*

29 Descriptive statistics were used to summarize data in IBM SPSS Statistics 25.0.0.1 for Windows (SPSS, Inc.,  
30 Chicago, IL). Values are median [interquartile range (IQR)], unless otherwise stated. A Wilcoxon signed-rank

1 test was used to compare DTend-exp, DTR and echogenicity between right and left side. In absence of left-to-  
2 right differences, the overall DTend-exp, DTR and diaphragm echogenicity in each patient were calculated by  
3 averaging the right and left side. The Friedman test was used to test if outcomes changed during 1.5-year  
4 follow-up. Spearman's correlation was used to assess correlations between age and MFM-20/32, and respiratory  
5 function and respiratory muscle strength tests. The correlation coefficient was considered moderate ( $r = 0.40$ –  
6  $0.59$ ), strong ( $r = 0.60$ – $0.80$ ), or very strong ( $r = 0.80$ – $1.0$ ). A  $p$ -value $<0.05$  was considered statistically  
7 significant.

8

### 9 **3. Results**

#### 10 *3.1 Patient characteristics*

11 Twenty-six LAMA2-MD patients (8 males, 18 females) with a median age of 21 [9; 31] (range 3-50) years and  
12 a median MFM-20/32 score of 32% [18; 70] were included. Eight (31%) patients were ambulant at the time of  
13 the inclusion. Nine patients (33%) had ever reached ambulatory status in their life, i.e. had a limb-girdle  
14 muscular dystrophy (LGMD) phenotype, while the remaining patients (66%) had never reached ambulatory  
15 status, i.e. had a congenital muscular dystrophy phenotype. All LAMA2-MD patients had a rigid spine. Four  
16 patients (three female, one male) did not have the full follow-up due to cardiorespiratory arrest (two hospital  
17 visits), burden of participation (one hospital visit, two home visits), personal circumstances (three home visits)  
18 and late inclusion (one hospital visit). Eleven SELENON-RM patients (8 males, 3 females) with a median age of  
19 20 [10; 33] (range 3-42) years and a median MFM-20/32 score of 76% [55; 82] participated in this study. Nine  
20 (82%) SELENON-RM patients were ambulant. All SELENON-RM patients had a rigid spine. One patient that  
21 participated through a home visit (male, 39 years) was lost from follow-up after the first visit due to the burden  
22 of participating in this study. Key clinical characteristics can be found in Table 1. A more detailed overview on  
23 the clinical features has been previously described<sup>(5)</sup> (Bouman et al. 2023, in press).

24

#### 25 *3.2 Medical history*

##### 26 *3.2.1 LAMA2-MD*

27 At baseline and at follow-up eight (31%) LAMA2-MD patients had non-invasive nocturnal mechanical  
28 ventilation and one (3.8%) (male, 21 years) had continuous invasive mechanical ventilation. Nine (35%)  
29 patients had respiratory infections before they participated in our study, mostly at pediatric age, of whom eight  
30 required admission to the hospital for temporary additional non-invasive or invasive mechanical ventilation and

1 antibiotics. One patient (female, 13 years) needed temporary support from an extracorporeal membrane  
2 oxygenation (ECMO) machine after infection with respiratory syncytial virus at pre-school age. During the  
3 follow-up period of this study, two (7.7%) patients (female, 3 years; male, 22 years) had several admissions to  
4 the intensive care due to bacterial and viral respiratory infections. None of the patients that reported to have  
5 been infected with SARS-CoV-2 needed additional treatments or hospital admission (vaccination coverage  
6 unknown). At baseline three (12%) patients indicated to suffer from dyspnea at some moment during a regular  
7 day: one patient (male, 22 years) with continuous mechanical ventilation when doing tasks that need  
8 concentration while sitting upright in the chair, and two patients (male, 27 and 23 years) with nocturnal non-  
9 invasive mechanical ventilation while taking a rest in the supine position. After 1.5-year follow-up, two  
10 additional patients indicated dyspnea: one patient (female, 11 years) without mechanical ventilation while  
11 walking and one patient (female, 14 years) with nocturnal non-invasive mechanical ventilation while wearing a  
12 cloth face mask in the upright position.

### 14 3.2.2 SELENON-RM

15 At baseline, eight (73%) patients and after 1.5 year follow-up nine (90%) patients were in need of non-invasive,  
16 nocturnal mechanical ventilation. In addition, one of them (female, 30 years) used positive air pressure (mouth  
17 piece ventilation) during the day. In medical history, three patients reported a respiratory infection that required  
18 hospital admission. During follow-up, none of the patients needed antibiotics or hospital admission due to  
19 respiratory infection. At baseline seven (64%) patients indicated dyspnea, all but one using non-invasive  
20 mechanical support, mostly while speaking or with physical exercise. No patient developed new dyspnea at a  
21 regular day during the 1.5-year follow-up.

### 23 3.3 X-ray of the spine

#### 24 3.3.1 LAMA2-MD

25 At baseline, 15 (71%) patients had scoliosis, of whom seven had undergone scoliosis surgery. Five patients had  
26 a scoliosis that was classified as mild, five as moderate and five as severe (Fig. 1). Seventeen patients had a  
27 second X-ray of the spine after one year follow-up showing scoliosis in 14 patients, of whom 13 patients already  
28 had a scoliosis at baseline. One patient (male, 8 years) developed a new, mild scoliosis and another patient  
29 (male, 14 years) progressed from a moderate to a severe scoliosis.

30

### 1 3.3.2 SELENON-RM

2 At baseline, seven (70%) patients had scoliosis, of whom four patients had undergone scoliosis surgery. Two  
3 patients had a scoliosis that was classified as mild, three as moderate and two as severe (Fig. 1). All patients had  
4 a second X-ray of the spine after one-year follow-up showing scoliosis in eight patients, including one patient  
5 who developed a new, mild scoliosis during follow-up. Further, one patient progressed from a mild to a  
6 moderate scoliosis and one patient progressed from moderate to severe scoliosis.

### 8 3.4 Spirometry

9 A full overview of spirometry, respiratory muscle strength tests and ultrasound of the diaphragm and intercostal  
10 muscles can be found in Table 2, Table 3 and Fig. 2.

#### 12 3.4.1 LAMA2-MD

13 At baseline, spirometry was performed in 20 patients. One patient was too young to perform spirometry (female,  
14 3 years), one patient refrained from spirometry due to complaints of fatigue (female, 31 years) and four patients  
15 were seen through home visits (two males, age 27 and 22 and two females, age 22 and 24 years). Seventeen  
16 (85%) patients had an impaired respiratory function. Median FVC% was 59% [33; 68], median FEV1% was  
17 64% [34; 71] and median FEV1/FVC was 0.90 [0.86; 0.95], indicating a restrictive pattern. PCF was abnormal  
18 in 15 (75%) patients (148 L/min [124.5; 295.8]). One patient (male, 42 years) had an abnormal dVC%. At  
19 baseline, the median FVC% was 65 [55; 94] in patients without a scoliosis, 64 [56; 71] with a mild scoliosis, 45  
20 [23; 73] with a moderate scoliosis and 19 [15; 24] with a severe scoliosis. At 1.5-year follow-up, spirometry was  
21 performed in 18 patients, of whom 14 (78%) patients had an impaired respiratory function and no patients  
22 developed new respiratory impairment. FVC%, FEV1% and dVC% did not change during 1.5-year follow-up  
23 period. PCF changed significantly during 1.5-year follow-up (148 L/min [125; 296] at baseline and 191 L/min  
24 [135; 309] after 1.5 year,  $p=0.003$ ).

#### 26 3.4.2 SELENON-RM

27 Spirometry was performed in 9 patients at baseline. One patient was too young to perform spirometry (male, 3  
28 years) and one patient was seen through home visits (male, 39 years). Respiratory function was impaired in all  
29 patients, with a median FVC% of 34% [31; 46], median FEV1% of 37% [33; 50] and median FEV1/FVC of  
30 0.94 [0.91; 0.96], indicating a restrictive pattern. PCF was abnormal in 8 (80%) patients (181 L/min [147; 230]).

1 Seven patients had an abnormal dVC% from the upright to the supine position. At baseline, the two patients  
2 without a scoliosis had a FVC% of 67% and 34%, the two patients with a mild scoliosis had a FVC% of 37%  
3 and 38%, and the three patients with a moderate scoliosis had a FVC% of 31 [30; 48], and the two patients with  
4 a severe scoliosis had a FVC% of 33% and 21%. At 1.5-year year follow-up, respiratory function was still  
5 impaired in all patients and all patients had an abnormal dVC%. FVC% and FEV1% significantly decreased  
6 during the 1.5-year follow-up (FVC% 34% [31; 46] at baseline and 31% [23; 41] after 1.5 year,  $p=0.003$ ;  
7 FEV1% 37% [33; 50] at baseline and 33% [24; 43] after 1.5 year,  $p=0.004$ ). The PCF did not change during 1.5-  
8 year follow-up (181 L/min [147; 230] at baseline and 198 L/min [186; 234] after 1.5 year,  $p>0.05$ ).

9

### 10 *3.5 Respiratory muscle strength*

11 There were no differences in MIP, MEP and SNIP between baseline and after 1.5-year follow-up for LAMA2-  
12 MD and SELENON-RM patients.

13

### 14 *3.6 Ultrasound of the diaphragm and intercostal muscles*

#### 15 *3.6.1 LAMA2-MD*

16 There was no difference in DTend-exp, DTR and echogenicity between the right and the left side of the  
17 diaphragm at all visits. At baseline, median DTend-exp was 1.4 [1.0; 1.5] mm, with a median z-score of 0.0 [-  
18 0.7; 0.4] and no patients with  $z\text{-score}<-2$  except for one patient (male, 42 years) with no identifiable diaphragm.  
19 On group level DTR was normal (2.2 [1.8; 2.7]), but four patients had a reduced DTR ( $<1.6$ ). Median  
20 diaphragm echogenicity z-score at baseline was 1.7 [0.6; 3.2]. Eight patients had an elevated echogenicity z-  
21 score, which indicates fibrosis or fat infiltration of the diaphragm. Echogenicity of the diaphragm could not be  
22 measured in four patients due to DTend-exp $<1$  mm on the left and/or right side. There was no change in Dtend-  
23 exp, DTmax-insp, DTR, echogenicity and ITend-exp during 1.5-year follow-up.

24

#### 25 *3.6.2 SELENON-RM*

26 There was no difference in DTend-exp and DTR between the right and the left side of the diaphragm at all  
27 visits. Median diaphragm thickness was 0.5 [0.3; 0.6] mm at baseline, with a median z-score of -2.5 [-3.1; -2.1],  
28 with nine patients having a  $z\text{-score}<-2$ . Median DTR was 1.2 [1.0; 1.7], with seven patients having a reduced  
29 DTR (Fig. 3). All patients had a DTend-exp $<1$  mm, so diaphragm echogenicity could not be measured. There

1 was no change in Dtend-exp, DTmax-insp and DTR during 1.5-year follow-up. ITend-exp changes significantly  
2 over time (2.9 [2.6; 3.6] at baseline and 3.0 [2.4; 4.2] after 1.5 year follow-up,  $p = 0.026$ ).

3

### 4 *3.7 Correlations between spirometry, respiratory muscle strength and motor function*

5 An overview on the observed correlations can be found in Table 4.

6

#### 7 3.7.1 LAMA2-MD

8 In all visits, MFM-20/32 was correlated with FVC%, MIP, MEP and SNIP. There were no correlations between  
9 age and MIP, MEP and SNIP in all visits, and no correlations between age and FVC% and dVC% in three out of  
10 four visits.

11

#### 12 3.7.2 SELENON-RM

13 Age was correlated with FVC%, dVC, MIP and SNIP in all visits. MFM-20/32 was correlated with FVC% and  
14 SNIP in three out of four visits.

15

## 16 **4. Discussion**

17 This study presents a 1.5-year prospective natural history study in patients with LAMA2-MD and SELENON-  
18 RM. The major findings of this study are: 1) need for noninvasive mechanical ventilation in a subgroup of  
19 LAMA2-MD patients and in the majority of SELENON-RM patients, which was strikingly disproportionate to  
20 motor function in SELENON-RM; 2) impaired respiratory function in the majority of the LAMA2-MD patients  
21 and in all SELENON-RM patients; 3) progressive decline in respiratory function during 1.5-year follow-up in  
22 SELENON-RM patients; 4) a relatively preserved diaphragm thickness and thickening in LAMA2-MD and  
23 severe diaphragm atrophy in SELENON-RM patients. 5) strong correlations between motor function, and  
24 respiratory function and SNIP in both neuromuscular diseases; and 6) strong to very strong correlations between  
25 age, respiratory function, inspiratory muscle strength and diaphragm function in SELENON-RM patients. We  
26 discuss the main findings below.

27

### 28 4.1 LAMA2-MD

29 Respiratory function was impaired in the majority of the patients, without a deterioration during 1.5-year follow-  
30 up, and was proportionate to ambulatory status and limb muscle weakness. Moreover, nine (35%) LAMA2-MD

1 patients were in need of mechanical ventilation. Remarkable was the large variability in respiratory function and  
2 diaphragm function in the cohort of LAMA2-MD patients, ranging from normal to severely impaired. This is  
3 congruent with the large variability in motor function and has previously been explained by the variable levels  
4 of merosin expression<sup>(31)</sup>. Our findings are in line with a previous study in LAMA2-MD patients that showed no  
5 yearly decrease in FVC% and only a minority of the patients with a decrease in FVC between the sitting and  
6 supine position<sup>(3, 17)</sup>. A linear annual decline in FVC% of 2.9% found in severely affected LAMA2-MD patients  
7 in another study cannot be translated to our cohort since we included patients with a variable disease severity  
8 based on the MFM-20/32 scores, ambulatory status and age at onset of symptoms<sup>(2)</sup>. Further, respiratory  
9 function was correlated to motor function in our cohort. A similar correlation has been described in spinal  
10 muscular atrophy<sup>(32, 33)</sup>, and to a lower extent in nemaline myopathies<sup>(26)</sup> and facioscapulohumeral dystrophy<sup>(34)</sup>.  
11 We did not correlate respiratory function (FVC%) to the level of merosin expression for multiple reasons. We  
12 did not perform muscle biopsies as part of research due to the burden of this procedure, and the number of  
13 patients in whom muscle biopsies with merosin staining was performed in clinical care was limited. In the  
14 available muscle biopsies, we were not able to perform reliable quantification of the level of merosin expression  
15 since merosin staining was frequently faded due to the long time interval. Further, the level of merosin  
16 expression is not dichotomous, but is a continuous scale, and dividing LAMA2-MD patients into solely two  
17 subgroups ‘complete’ or ‘partial’ merosin deficiency is artificial. There was no cross-sectional correlation  
18 between age and respiratory function. A likely explanation is that LAMA2-MD patients with severe respiratory  
19 impairment have a limited life expectancy and mostly do not survive after the third decade<sup>(16)</sup>. Consequently, the  
20 cohort of patients, especially at an older age, is biased towards the less severely affected patients. This could  
21 give the wrong impression of a stabilization or improvement of respiratory function with increasing age.  
22 Moreover, we observed that respiratory function was lower in patients with a more severe scoliosis. We did not  
23 perform statistical analysis on respiratory function and severity of scoliosis due to the small subgroups. Scoliosis  
24 is known to alter respiratory mechanics in neuromuscular diseases and thereby impairing respiratory function<sup>(2,</sup>  
25 <sup>35)</sup>. . Low MEP and low PCF indicate prominent expiratory muscle weakness, which leads to ineffective cough,  
26 increasing the risk of respiratory complications including respiratory infections. We consider the variability in  
27 PCF during the 1.5-year follow-up as not relevant to assess respiratory disease progression since it is an absolute  
28 value, contrarily to other respiratory function variables that are corrected for age, length and weight (FVC%,  
29 FEV1%, dVC% and FEV1/FVC). Due to the absence of this correction, an increase in PCF might thus wrongly  
30 give the impression of an improvement in PCF.

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#### 4.2 SELENON-RM

The most noticeable feature in SELENON-RM was severe diaphragm atrophy that was already present at a young age. The youngest patient (male, 3 years) had severe diaphragm atrophy, yet did not need mechanical ventilation. He was able to compensate diaphragm dysfunction with activation of accessory respiratory muscles. In general, due to the severe diaphragm atrophy, caution should be paid to the interpretation of the DTR. Small absolute changes in measurements of DTend-exp and DTmax-insp could result in a relatively normal DTR, incorrectly giving the impression of a normal diaphragm function. This study showed a large dVC%, which is a good indication of diaphragm weakness, even in subjects who might have global inspiratory muscle dysfunction<sup>(1, 36)</sup>. We additionally found a low SNIP, which is predominantly dependent on diaphragm function<sup>(37, 38)</sup>. Our findings are in line with a previous study using esophageal and gastric pressure measurements in SELENON-RM patients showing that diaphragmic dysfunction is a characteristic feature<sup>(18)</sup>. Respiratory function was impaired in all patients, with a high need for mechanical ventilation (73% at baseline and 90% at follow-up), which was in line with a previously reported frequencies<sup>(39)</sup>. Respiratory function was strikingly disproportionate to motor function, with respiratory impairment leading to the need of mechanical ventilation in ambulant patients with relatively preserved limb muscle strength. Moreover, we observed that respiratory function was lower in patients with a more severe scoliosis. In SELENON-RM patients, thoracic deformities are known to contribute to respiratory failure<sup>(4)</sup>. Scoliosis and restrictive respiratory impairment were often diagnosed simultaneously at approximately the end of the first decade, and earlier scoliosis and respiratory failure required earlier non-invasive mechanical ventilatory support<sup>(4)</sup>. We additionally detected that respiratory function deteriorated during 1,5 year follow-up, despite stable motor function. This was in line with the findings of a retrospective natural history study on SELENON-RM showing an annual decrease in FVC% of -2.04% per year<sup>(39)</sup>. We also observed strong to very strong correlations between age and respiratory function, respiratory muscle strength and diaphragm function at all visits. Finally, during the follow-up period, mechanical ventilation was initiated in two additional patients (patient 4, 22 years and patient 10, 12 years), confirming the progressive nature of respiratory impairment. MEP and PCF were low in all visits, indicating prominent expiratory muscle weakness, which contributes to respiratory complications. ITend-exp changed significantly over time. However, due to small groups and varying absolute thickness, we consider this as an irrelevant change.



1 Altogether, we conclude that inspiratory and expiratory respiratory function is impaired in the majority of  
2 LAMA2-MD and all SELENON-RM patients, and that respiratory impairment is caused by a combination of  
3 diaphragm weakness, accessory inspiratory muscle weakness, expiratory muscle weakness and scoliosis<sup>(40)</sup>. Our  
4 study highlights severe diaphragm atrophy and diaphragm dysfunction in SELENON-RM patients starting at a  
5 young age. The study further confirms the characteristic, relatively fast progressive nature of respiratory  
6 impairment in SELENON-RM patients.

7

#### 8 *4.3 Recommendations for clinical care*

9 The consensus statements on congenital muscular dystrophies and congenital myopathies, and the clinical care  
10 recommendation for LAMA2-MD as published on *GeneReviews* provide a comprehensive overview on  
11 respiratory care<sup>(14, 15, 21)</sup>. Our study supports the expert opinion in these consensus statements and we suggest to  
12 implement these recommendations into the clinical care for LAMA2-MD and SELENON-RM. Respiratory  
13 function (FVC%, dVC; in upright and supine position) and respiratory muscle strength (SNIP; in upright  
14 position) should be performed at least one time per two years in LAMA2-MD patients and annually in  
15 SELENON-RM patients order to early detect respiratory impairment and prevent respiratory complications,  
16 independent of the motor function, and more often on indication based on clinical symptoms (dyspnea, headache  
17 when waking up). The interval of one year in SELENON-RM is chosen due to the progressive nature of  
18 respiratory function in SELENON-RM during the LAST STRONG Study. SNIP should additionally be  
19 performed in patients in whom diaphragm dysfunction is present or expected, consequently in all SELENON-  
20 RM patients. Since standing height is not reflective of lung growth in patients with scoliosis, serial respiratory  
21 function tests are needed to monitor patients over time. Furthermore, since complications during sleep usually  
22 precede abnormalities during wakefulness, regular assessment of nocturnal respiratory function through sleep  
23 studies is indicated. This is particularly needed in SELENON-RM patients due to their severe diaphragm  
24 dysfunction. Non-invasive positive pressure mechanical ventilation is the recommended modality in the  
25 treatment of chronic respiratory failure in neuromuscular diseases.

26

#### 27 *4.4 Recommendations for research*

28 We propose to use spirometry and respiratory muscle strength tests as clinical outcome measure in natural  
29 history studies and future clinical trials on possible treatment options. In particular FVC% and SNIP showed  
30 good correlations with motor function. Diaphragm ultrasound can help in diagnosis of diaphragm dysfunction in

1 patients with neuromuscular diseases. However, no change during 1.5-year follow-up was found, and therefore  
2 we consider ultrasound of the diaphragm not as the first choice for a clinical outcome measure. In patients < 5  
3 years that cannot perform spirometry, ultrasound of the diaphragm can nevertheless be used as clinical outcome  
4 measures to give an indication of diaphragm function.

5 We plan to extend the present 1.5-year natural history study to a follow-up of 3 and 5 years (extended LAST  
6 STRONG) to assess respiratory function on the longer term and to identify risk factors for developing  
7 respiratory impairment.

8

#### 9 *4.5 Strengths and limitations*

10 The major strengths of this study include its standardized and prospective design with an extensive number of  
11 clinically available respiratory function tests in an unselected cohort of LAMA2-MD and SELENON-RM  
12 patients, with great variability in age and disease severity. Further, we had a high participation rate and only a  
13 minor loss from follow-up. The number of patients in this study is nevertheless low, which is inherent to the low  
14 prevalence of these neuromuscular diseases. Therefore, future international collaborations are essential.  
15 Moreover, the youngest patients were 3 years old, leading to a knowledge gap in younger patients. Finally, a  
16 1.5-year follow-up is too short to detect minor changes in respiratory function and to determine risk factors for  
17 developing respiratory impairment on the long-term. Caution should be paid to the interpretation of spirometry  
18 and respiratory muscle strength tests in patients with neuromuscular diseases since major fluctuations within a  
19 patient might possibly be caused by fatigue or intercurrent respiratory infections<sup>(1)</sup>.

20

#### 21 **5. Conclusion**

22 Respiratory impairment was present in the majority of LAMA2-MD patients and in all SELENON-RM patients.  
23 In SELENON-RM, severe diaphragm dysfunction, which was strikingly disproportionate to ambulatory status  
24 and muscle limb weakness, was present in all patients. Spirometry and respiratory muscle strength tests are  
25 useful in respiratory assessment in clinical care and are proposed as clinical outcome measure for natural history  
26 studies and future clinical trials starting at a young age (5 years).

27

#### 28 **6. Statements and declarations**

##### 29 *6.1 Acknowledgements*

1 We thank all patients and their relatives for participation in our study. We thank Marit Boxum and Daniëlle  
2 Franken for their help in contacting patients and requesting medical data from other hospitals. Several authors of  
3 this publication are members of the Radboudumc Neuromuscular Center (Radboud-NMD), Netherlands  
4 Neuromuscular Center (NL-NMD) and European Reference Network for rare neuromuscular diseases (EURO-  
5 NMD).

## 6 7 *6.2 Funding*

8 The work was supported by a grant from Stichting Spieren voor Spieren, Stichting Stofwisselkracht and  
9 Stichting Voor Sara, The Netherlands. These did not have any influence in study design, in the collection,  
10 analysis and interpretation of data, in the writing of the report or in the decision to submit the article for  
11 publication.

## 12 13 *6.3 Competing interests*

14 The authors declare that they have no conflict of interest.

## 15 16 *6.4 Declaration of interest*

17 Declarations of interest: none

## 18 19 *6.5 Ethics approval and consent to participate*

20 This study was registered at clinicaltrials.gov (NCT04478981). This study was approved by the medical ethical  
21 reviewing committee of Region Arnhem-Nijmegen (NL-number NL64269.091.17, dossier number 2017-3911;  
22 date of approval of last amendment: 8 July 2020). From all patients, or in case of children their parent or legal  
23 guardian, informed consent has been obtained prior to inclusion in our study. The patients depicted in Fig. 3  
24 additionally provided consent for publication of their pictures.

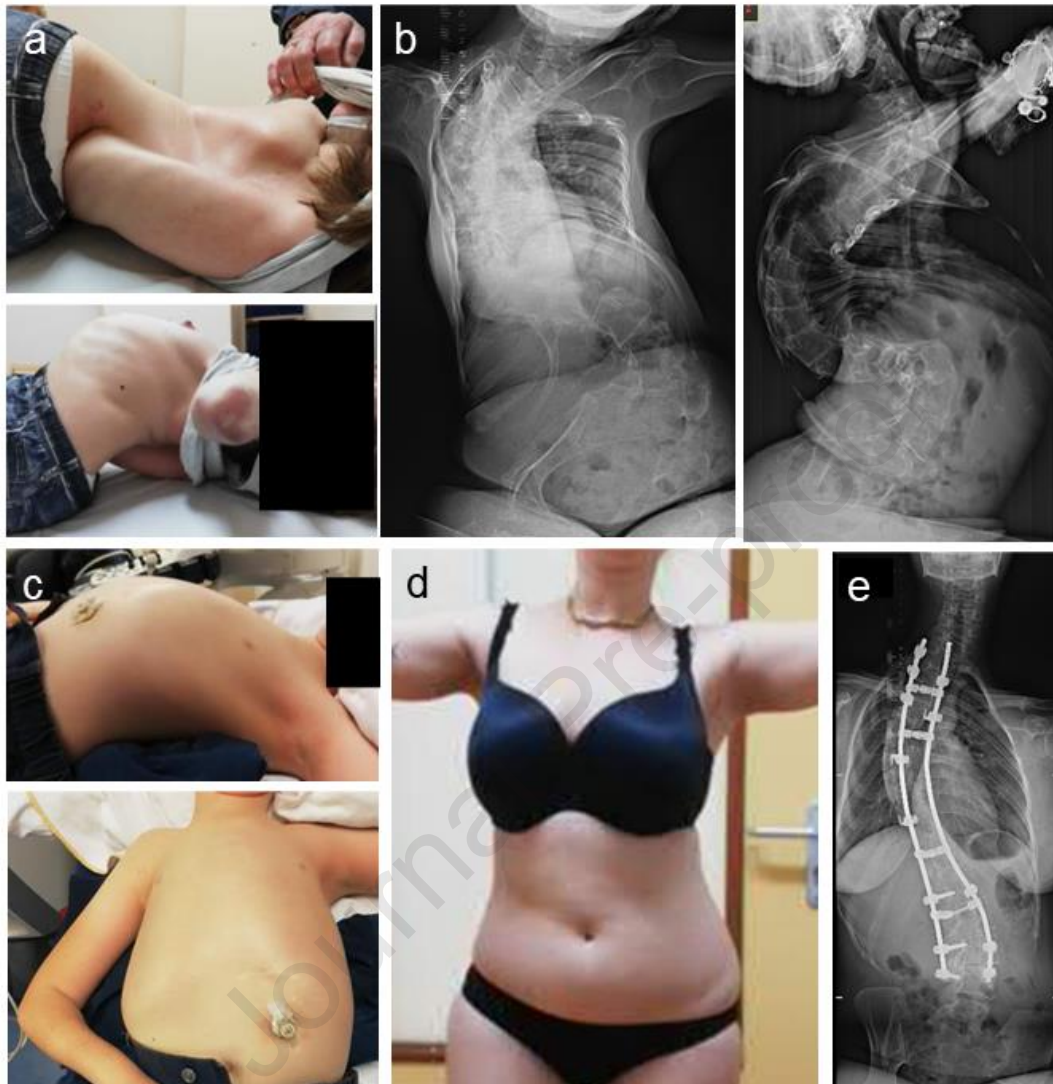
## 25 26 *6.6 Data availability statement*

27 The data that support the findings of this study are available within the article and supplementary material, and  
28 from the corresponding author, upon reasonable request.

## 29 30 *6.7 Authors' contribution*

1 KB: study concept design, inclusion of patients, (co-)performing all medical examinations, collection of all data  
2 and performing all (statistical) analyses, manuscript writing and revision. JD: study concept design, (co-  
3 )performing all medical examinations and collection of all data, critical revision of manuscript. JvD: manuscript  
4 writing and revision and performing (statistical) analyses. JG, BvE, CE and NV: study concept design and  
5 critical revision of manuscript. All authors read and approved the manuscript.  
6

Journal Pre-proof

1 **Figures**2 *No color needs to be used for any figures in print.*

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4

5 **Fig. 1 Thorax deformities in LAMA2-MD and SELENON-RM patients.** (a) severe thorax deformity in a

6 LAMA2-MD patient, showing an asymmetric protrusion of the anterior chest wall, an asymmetric retropulsion

7 of the posterior chest wall, a rotation component of the chest wall and scoliosis. (b) X-ray of the spine and

8 thorax of patient shown in a, showing a severe kyphoscoliotic spine with a Cobb's angle of 130 degrees between

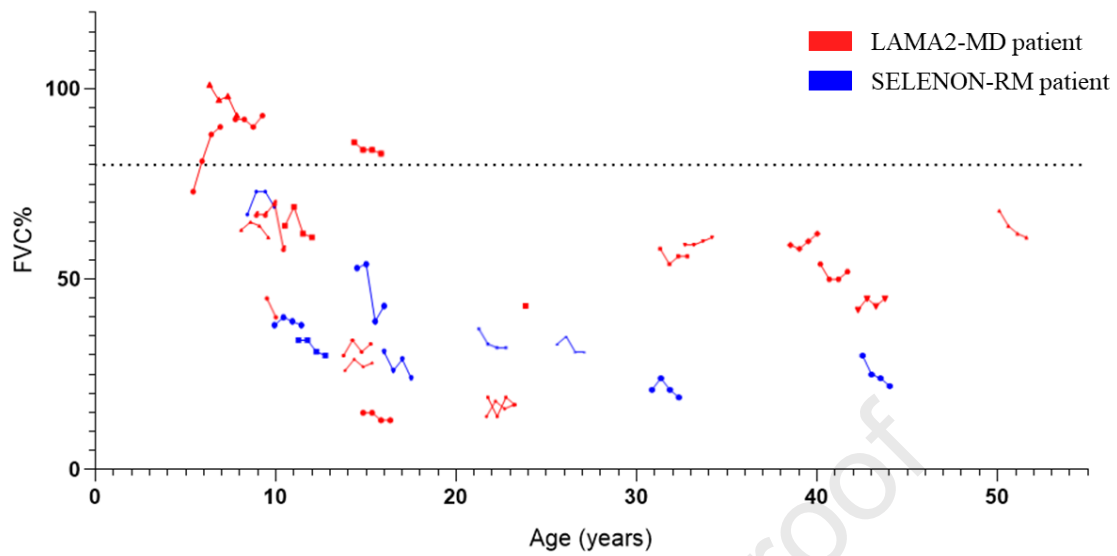
9 C7 and L2 and a Cobb's angle of 100 degrees between L2/L3. (c) severe thorax deformities showing pectus

10 carinatum. (d) SELENON-patient, showing from the outside only subtle thorax deformities. (e) X-ray of patient

11 shown in d showing evident thorax deformities secondary to scoliosis.

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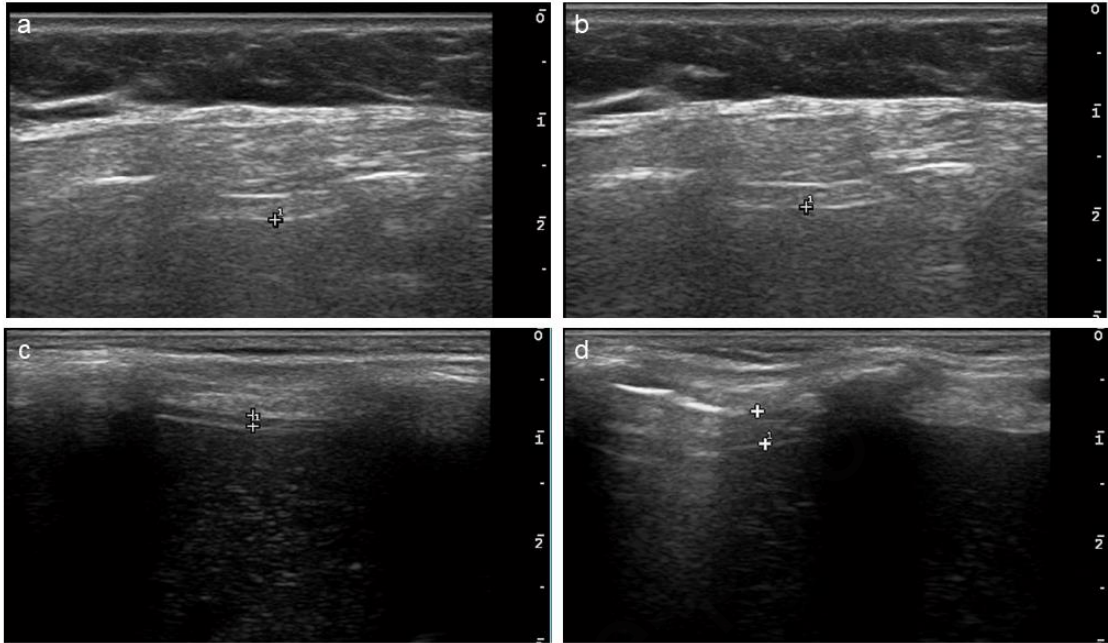
3 **Fig. 2 Overview on percentage predicted forced vital capacity (FVC%) in LAMA2-MD and SELENON-**

4 **RM patients versus age (years).** Each line represents one patient followed for 1.5 year. Y = 80% represent the

5 cut-off value for impaired lung function. FVC% = percentage predicted forced vital capacity.

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4 **Fig. 3 Ultrasound of the diaphragm in LAMA2-MD and SELENON-RM patient.** (a) Ultrasound of the  
 5 diaphragm of a SELENON-RM at end-expiration showing no identifiable diaphragm at end expiration. (b)  
 6 Ultrasound of the diaphragm of the same SELENON-RM patient showing no identifiable diaphragm at maximal  
 7 inspiration. The marker in images a and b indicates the position where the diaphragm should be located. Note,  
 8 that just above the marker a muscle layer is visible. However, through extensive scanning we identified this  
 9 muscle layer as the intercostal muscles and not the diaphragm. (c) Ultrasound of the diaphragm of a LAMA2-  
 10 MD patient showing a normal diaphragm thickness ( $DT_{\text{end-exp}}=1.0$  mm). (d) Ultrasound of the diaphragm of  
 11 the same LAMA2-MD patient with evident diaphragm thickening ( $DT_{\text{max-insp}}=3.2$  mm).

12

1 **Tables**2 **Table 1. demographic characteristics of patients with LAMA2-MD and SELENON-RM.**

Demographics	Baseline	After 1,5 year
<b>LAMA2-MD</b>		
Number of patients	26	22
Age at examination (years)	21 [9; 31]	20 [10; 33]
Males (%)	8 (31%)	8 (35%)
(non-)invasive mechanical ventilation	9 (35%)	7 (32%)
BMI – adult (kg/m <sup>2</sup> )	21 [18; 24]	21 [18; 25]
BMI – child (kg/m <sup>2</sup> )	17 [16; 22]	20 [15; 21]
Number of ambulant patients	8 (31%)	8 (35%)
MFM-20/32 total score (%)	32 [18; 70]	44 [14; 72]
MFM-20/32 D1	3 [2; 45]	10 [0; 44]
MFM-20/32 D2	36 [10; 81]	53 [10; 83]
MFM-20/32 D3	81 [57; 95]	88 [48; 96]
<b>SELENON-RM</b>		
Number of patients	11	10
Age at examination (years)	16 [9; 31]	16 [11; 28]
Males (%)	8 (73%)	7 (70%)
(non-)invasive mechanical ventilation	8 (73%)	9 (90%)
BMI – adult (kg/m <sup>2</sup> )	23 [20; 25]	23 [21; 26]
BMI – child (kg/m <sup>2</sup> )	15 [14; 20]	15 [13; 21]
Number of ambulant patients	9 (82%)	8 (80%)
MFM-20/32 total score	76 [55; 82]	71 [59; 81]
MFM-20/32 D1	59 [21; 63]	50 [23; 66]
MFM-20/32 D2	83 [69; 86]	81 [76; 85]
MFM-20/32 D3	95 [95; 95]	95 [95; 100]

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4 *LAMA2-MD = LAMA2-related muscular dystrophy; SELENON-RM = SELENON-related congenital myopathy;*5 *BMI = body mass index; MFM-20/32 = motor function measurement 20/32; MFM-20/32 D1 = motor function*6 *measurement 20/32 domain 1, standing and transfers; MFM-20/32 D2 = motor function measurement 20/32*7 *domain 2, axial and proximal motor function; MFM-20/32 D3 = motor function measurement 20/32 domain 3,*8 *distal motor function. Data is presented as median [p25; p75] or as n (%).*

9



1 **Table 2. spirometry, respiratory muscle strength and ultrasound of the diaphragm and intercostal**  
 2 **muscles in LAMA2-MD.**

LAMA2-MD					
	Baseline (n = 20)	6 months (n = 19)	12 months (n = 18)	18 months (n = 18)	P value
<b>Spirometry</b>					
FVC%	59 [33; 68]	58 [34; 69]	60 [30; 74]	60 [32; 67]	ns
FEV1%	64 [34; 71]	63 [34; 70]	61 [32; 71]	61 [31; 68]	ns
dVC%	2.2 [-1.0; 6.5]	0.7 [-9.4; 7.7]	0.6 [-7.8; 4.9]	-3.7 [-8.2; 3.0]	ns
FEV1/FVC	0.90 [0.86; 0.95]	0.89 [0.86; 0.91]	0.90 [0.84; 0.95]	0.87 [0.85; 0.93]	ns
PCF (L/min)	148 [125; 296]	170 [118; 295]	199 [138; 315]	191 [135; 309]	0.003
<b>Respiratory muscle strength</b>					
	Baseline (n = 25)	6 months (n = 24)	12 months (n = 23)	18 months (n = 21)	
MIP (cmH <sub>2</sub> O)	49 [29; 63]	43 [28; 58]	50 [25; 62]	52 [25; 64]	ns
MEP (cmH <sub>2</sub> O)	29 [20; 52]	42 [21.; 68]	49 [25; 63]	45 [27; 66]	ns
SNIP (cmH <sub>2</sub> O)	40 [28; 60]	40 [16; 60]	36 [23; 46]	48 [33; 66.5]	ns
<b>Diaphragm ultrasound</b>					
	Baseline (n = 22)	6 months (n = 20)	12 months (n = 19)	18 months (n = 18)	
DTend-exp (mm)	1.4 [1.0; 1.5]	1.1 [1.0; 1.3]	1.2 [1.1; 1.4]	1.2 [1.0; 1.2]	ns
DTend-exp (z-score)	0.0 [-0.7; 0.4]*	-0.5 [-0.9; -0.1]	-0.3 [-0.6; -0.2]*	-0.6 [-0.8; -0.3]*	ns
DTR	2.2 [1.8; 2.7]	2.2 [1.8; 2.9]	2.3 [1.8; 2.7]	2.3 [1.8; 2.9]	ns
Echogenicity (z-scores)	2.0 [0.8; 3.3]	2.1 [1.0; 3.1]	1.7 [0.4; 3.2]	2.0 [0.9; 3.1]	ns
<b>Intercostal muscle ultrasound</b>					
ITend-exp (mm)	2.4 [2.0; 2.9]	2.5 [2.1; 3.1]	2.3 [1.7; 3.3]	2.0 [1.6; 3.0]	ns

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4 \* z-score could not be calculated in one patient since diaphragm was not identifiable

5

6 LAMA2-MD = LAMA2-related muscular dystrophy; FVC% = percentage predicted of forced vital capacity;

7 FEV1% = percentage predicted of forced expiratory volume in the first second; dVC% = percentage decrease

8 in vital capacity from the upright to the supine position; PCF = peak cough flow; MIP = maximal inspiratory

9 pressure; MEP = maximal expiratory pressure; SNIP = sniff nasal inspiratory pressure; DTend-exp =

10 diaphragm thickness end expiratory; DTR = diaphragm thickness ratio; ITend-exp = intercostal muscle

11 thickness end-expiratory. Data is presented as median [p25; p75].

12

1 **Table 3. spirometry, respiratory muscle strength and ultrasound of the diaphragm and intercostal**  
 2 **muscles in SELENON-RM patients**

SELENON-RM					
	Baseline (n = 9)	6 months (n = 9)	12 months (n = 9)	18 months (n = 9)	P value
<b>Spirometry</b>					
FVC (% predicted)	34 [31; 46]	34 [26; 47]	31 [27; 39]	31 [23; 41]	0.003
FEV1 (% predicted)	37 [33; 50]	37 [28; 50]	33 [28; 41]	33 [24; 43]	0.004
dVC%	30 [7.7; 41]	35 [14; 47]	27 [21; 37]	20 [15; 35]	ns
FEV1/FVC	0.94 [0.91; 0.96]	0.93 [0.90; 0.94]	0.90 [0.85; 0.93]	0.91 [0.90; 0.95]	ns
PCF (L/min)	181 [147; 230]	186 [166; 213]	188 [175; 217]	198 [186; 234]	ns
<b>Respiratory muscle strength</b>					
	Baseline (n = 10)	6 months (n = 9)	12 months (n = 9)	18 months (n = 9)	
MIP (cmH <sub>2</sub> O)	34 [26; 40]	34 [27; 47]	30 [25; 42]	27 [24.5; 41]	ns
MEP (cmH <sub>2</sub> O)	50 [43; 62]	52 [37; 65]	50 [43; 65]	56 [43; 72]	ns
SNIP (cmH <sub>2</sub> O)	37 [17; 44]	32 [22; 40]	31 [26; 37]	33 [21; 38]	ns
<b>Diaphragm ultrasound</b>					
	Baseline (n = 10)	6 months (n = 10)	12 months (n = 10)	18 months (n = 10)	
DTend-exp (mm)	0.5 [0.3; 0.6]	0.4 [0.1; 0.6]	0.2 [0; 0.6]	0.2 [0; 0.5]	ns
DTend-exp (z-score)	-2.5 [-3.1; -2.1]	-2.6 [-3.3; -2.0]*	-2.3 [-3.6; -1.7]**	-1.7 [-2.6; -1.5]***	ns
DTR	1.2 [1.0; 1.7]	1.2 [1.1; 1.7]	1.2 [1.0; 2.0]	1.1 [1.0; 1.3]	ns
Echogenicity	Not applicable, all patients had DTend-exp < 1 mm in all visits				n.a.
<b>Intercostal muscle ultrasound</b>					
ITend-exp (mm)	2.9 [2.6; 3.6]	2.2 [1.7; 2.9]	2.6 [1.8; 3.4]	3.0 [2.4; 4.2]	0.026

3

4 \* z-score could not be calculated in one patient since diaphragm was not identifiable

5 \*\* z-score could not be calculated in four patients since diaphragm was not identifiable

6 \*\*\* z-score could not be calculated in five patients since diaphragm was not identifiable

7

8 *SELENON-RM = SELENON-related congenital myopathy; FVC% = percentage predicted of forced vital*9 *capacity; FEV1% = percentage predicted of forced expiratory volume in the first second; dVC% = percentage*10 *decrease in vital capacity from the upright to the supine position; PCF = peak cough flow; MIP = maximal*11 *inspiratory pressure; MEP = maximal expiratory pressure; SNIP = sniff nasal inspiratory pressure; DTend-exp*12 *= diaphragm thickness end expiratory; DTR = diaphragm thickness ratio; ITend-exp = intercostal muscle*13 *thickness end-expiratory. Data is presented as median [p25; p75].*

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1 **Table 4. Correlations between spirometry, respiratory muscle strength and motor function measurement**2 **20/32.**

		LAMA2-MD					
	Visit	MFM-20/32 (%)	FVC%	dVC (%)	MIP (mmH <sub>2</sub> O)	MEP (mmH <sub>2</sub> O)	SNIP (mmH <sub>2</sub> O)
Age (years)	1	-0.173	0.390	0.106	0.073	0.241	-0.249
	2	-0.143	-0.485	0.318	0.045	0.100	-0.136
	3	-0.135	-0.526*	0.158	-0.182	0.111	-0.352
	4	-0.164	-0.422	0.557*	-0.145	0.216	-0.123
MFM-20/32 (%)	1	-	0.783**	-0.093	0.716**	0.724**	0.810**
	2		0.784**	-0.115	0.751**	0.752**	0.844**
	3		0.709**	0.257	0.748**	0.696**	0.849**
	4		0.795**	0.308	0.813**	0.647**	0.524*
		SELENON-RM					
Age (years)	1	-0.542	-0.867**	0.950**	-0.733*	0.614	-0.689*
	2	-0.718*	-0.833**	0.683*	-0.717*	0.283	-0.729*
	3	-0.479	-0.824**	0.917**	-0.803**	0.126	-0.932**
	4	-0.413	-0.800**	0.683*	-0.812**	-0.167	-0.711*
MFM-20/32 (%)	1	-	0.669*	-0.418	0.450	-0.015	0.768**
	2		0.695*	-0.407	0.237	0.153	0.828**
	3		0.740*	-0.450	0.293	0.109	0.271
	4		0.552	-0.226	0.282	0.427	0.693*

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5 *LAMA2-MD = LAMA2-related muscular dystrophy; SELENON-RM = SELENON-related congenital myopathy;*6 *FVC = forced vital capacity; MIP = maximal inspiratory pressure; MEP = maximal expiratory pressure; SNIP*7 *= sniff nasal inspiratory pressure; MFM-20/32 = motor function measurement 20/32; \* =  $p < 0.05$ ; \*\* =  $p <$* 8 *0.01.*

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1 **References**

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**Study highlights**

- The majority of LAMA2-MD and all SELENON-RM patients have respiratory impairment
- This is caused by diaphragm weakness, accessory muscle weakness and scoliosis
- In SELENON-RM, respiratory impairment was progressive and disproportionate
- SELENON-RM patients had a striking diaphragm atrophy and dysfunction at a young age
- Respiratory testing is useful in clinical care and as outcome measure in trials

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**Conflict of Interest**

The Conflicts of Interest from any individual author can be found below.

Karlijn Bouman	none
Jeroen L.M. van Doorn	none
Jan T. Groothuis	none
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