

University of Groningen

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

Bouman, Karlijn; van Doorn, Jeroen L M; Groothuis, Jan T; Wijkstra, Peter J; van Engelen, Baziël G M; Erasmus, Corrie E; Doorduyn, Jonne; Voermans, Nicol C

Published in:
European Journal of Paediatric Neurology

DOI:
[10.1016/j.ejpn.2023.11.005](https://doi.org/10.1016/j.ejpn.2023.11.005)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Final author's version (accepted by publisher, after peer review)

Publication date:
2023

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Bouman, K., van Doorn, J. L. M., Groothuis, J. T., Wijkstra, P. J., van Engelen, B. G. M., Erasmus, C. E., Doorduyn, J., & Voermans, N. C. (in press). Respiratory function in LAMA2-related muscular dystrophy and SELENON-related congenital myopathy, a 1.5-year natural history study. *European Journal of Paediatric Neurology*, 48, 30-39. <https://doi.org/10.1016/j.ejpn.2023.11.005>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

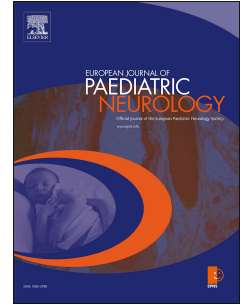
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Journal Pre-proof

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

Karlijn Bouman, Jeroen L.M. van Doorn, Jan T. Groothuis, Peter J. Wijkstra, Baziel G.M. van Engelen, Corrie E. Erasmus, Jonne Doorduyn, Nicol C. Voermans



PII: S1090-3798(23)00167-8

DOI: <https://doi.org/10.1016/j.ejpn.2023.11.005>

Reference: YEJPN 3108

To appear in: *European Journal of Paediatric Neurology*

Received Date: 5 October 2023

Revised Date: 10 November 2023

Accepted Date: 12 November 2023

Please cite this article as: Bouman K, van Doorn JLM, Groothuis JT, Wijkstra PJ, van Engelen BGM, Erasmus CE, Doorduyn J, Voermans NC, Respiratory function in LAMA2-related muscular dystrophy and SELENON-related congenital myopathy, a 1.5-year natural history study, *European Journal of Paediatric Neurology* (2023), doi: <https://doi.org/10.1016/j.ejpn.2023.11.005>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2023 Published by Elsevier Ltd on behalf of European Paediatric Neurology Society.

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

3

4 Karlijn Bouman, MD^{a,b}, Jeroen L.M. van Doorn, MSc^b, Jan T. Groothuis MD, PhD^c, Peter J. Wijkstra, MD,
5 PhD^d, Baziel G.M. van Engelen, MD, PhD^b, Corrie E. Erasmus MD, PhD^a, Jonne Doorduyn PhD^{b*}, Nicol C.
6 Voermans, MD, PhD^{b*}

7

8

9 * contributed equally

10

11 ^aDepartment of Pediatric Neurology, Donders Institute for Brain, Cognition and Behaviour, Amalia Children's
12 Hospital, Radboud university medical center, Nijmegen, The Netherlands

13 ^bDepartment of Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud university medical
14 center, Nijmegen, The Netherlands

15 ^cDepartment of Rehabilitation, Donders Institute for Brain, Cognition and Behaviour, Radboud university
16 medical center, Nijmegen, The Netherlands

17 ^dDepartment of Pulmonary Diseases and Home Mechanical Ventilation, University Medical Centre Groningen,
18 Groningen, The Netherlands.

19

20 **Corresponding author:** Karlijn Bouman, MD, Department of Pediatric Neurology and Neurology,
21 Radboudumc, Geert Grooteplein Zuid 10, 6525 GA, Nijmegen, The Netherlands. Tel. 0031629677753.

22 Karlijn.bouman@radboudumc.nl. ORCID number: 0000-0003-4964-3077

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⁽¹⁻⁵⁾ (Bouman et al. 2023, in press). LAMA2-MD and SELENON-
6 RM are caused by pathologic variants in the *LAMA2* and *SELENON (SEPNI)* genes, coding for the laminin
7 alpha2 subunit and selenoprotein N, respectively^(6,7). No curative therapies exist but promising preclinical trials
8 are ongoing. These expected trials emphasize the need to reach trial readiness⁽⁸⁻¹³⁾. 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^(14,15).

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^(1,2,16). 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⁽³⁾. 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⁽¹⁶⁾. 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⁽³⁾.
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^(3,17). 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⁽¹⁾.
23 Weakness of inspiratory and expiratory muscles, scoliosis and thoracic deformities contribute to respiratory
24 failure⁽¹⁾. 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⁽¹⁸⁾.

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^(19,20). 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^(14, 15, 21). 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*⁽²²⁾. 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.

12

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⁽²³⁾. 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⁽²⁴⁾.
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 ≥ 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)⁽²⁵⁾. Restrictive pulmonary function was defined as FVC $<80\%$ ⁽²⁶⁾. Peak cough flow (PCF) was
14 assessed in the sitting position and was considered abnormal if <270 l/min⁽²⁷⁾. 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 $>10\%$ ⁽¹⁷⁾. 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 ≥ 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^(19, 20, 28). 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⁽²⁹⁾. 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 8th or 9th 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⁽²⁹⁾.

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)⁽²³⁾. 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 3rd visit, and
20 was dichotomized as present (Cobb Angle ≥ 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⁽³⁰⁾. 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⁽⁵⁾ (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.

13

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.

22

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⁽³¹⁾. 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^(3, 17). 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⁽²⁾. Further, respiratory
9 function was correlated to motor function in our cohort. A similar correlation has been described in spinal
10 muscular atrophy^(32, 33), and to a lower extent in nemaline myopathies⁽²⁶⁾ and facioscapulohumeral dystrophy⁽³⁴⁾.
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⁽¹⁶⁾. 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^{(2,}
25 ³⁵⁾. . 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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

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^(1, 36). We additionally found a low SNIP, which is predominantly dependent on diaphragm function^(37, 38). 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⁽¹⁸⁾. 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⁽³⁹⁾. 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⁽⁴⁾. 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⁽⁴⁾. 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⁽³⁹⁾. 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⁽⁴⁰⁾. 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.

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^(14, 15, 21). 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.

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⁽¹⁾.

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.

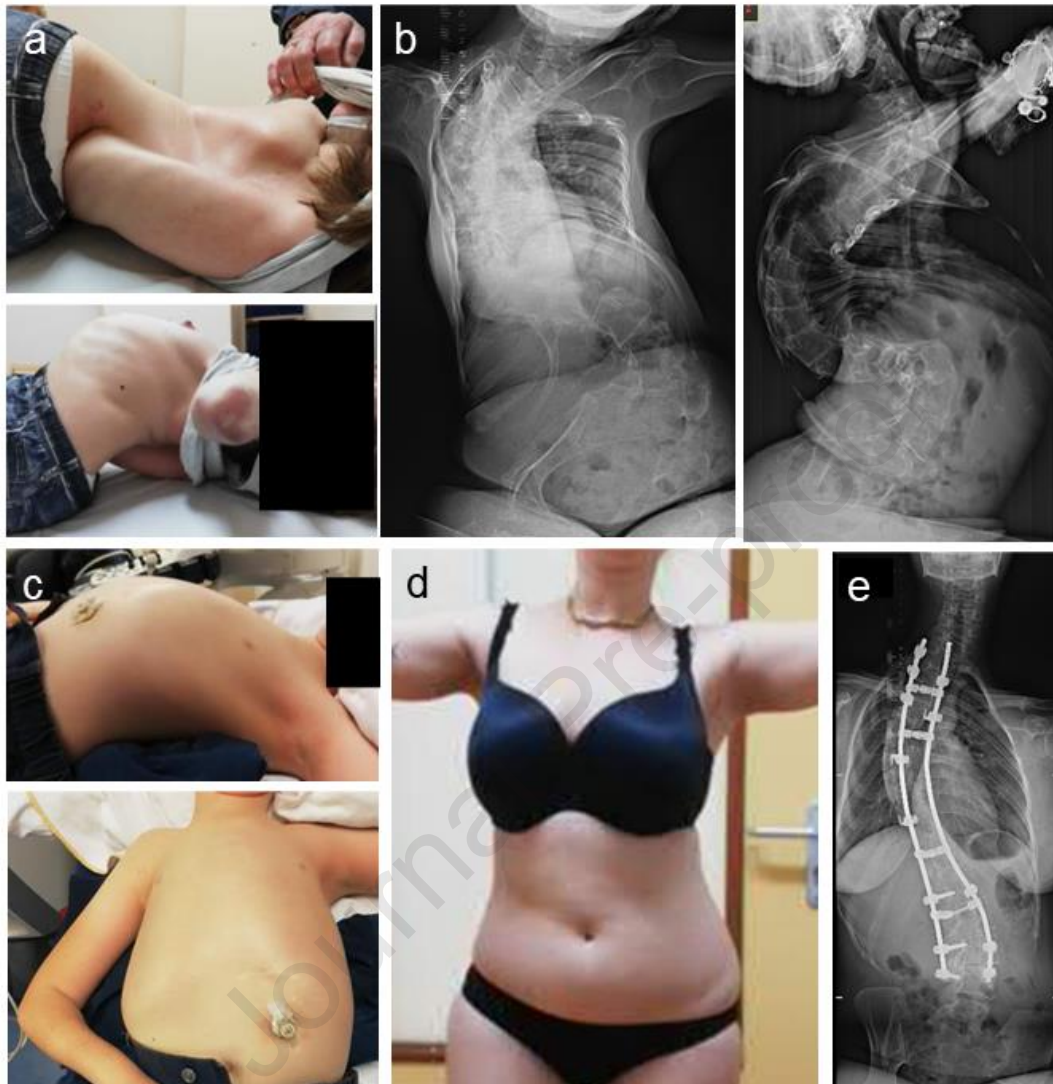
25 26 *6.6 Data availability statement*

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.*

3

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

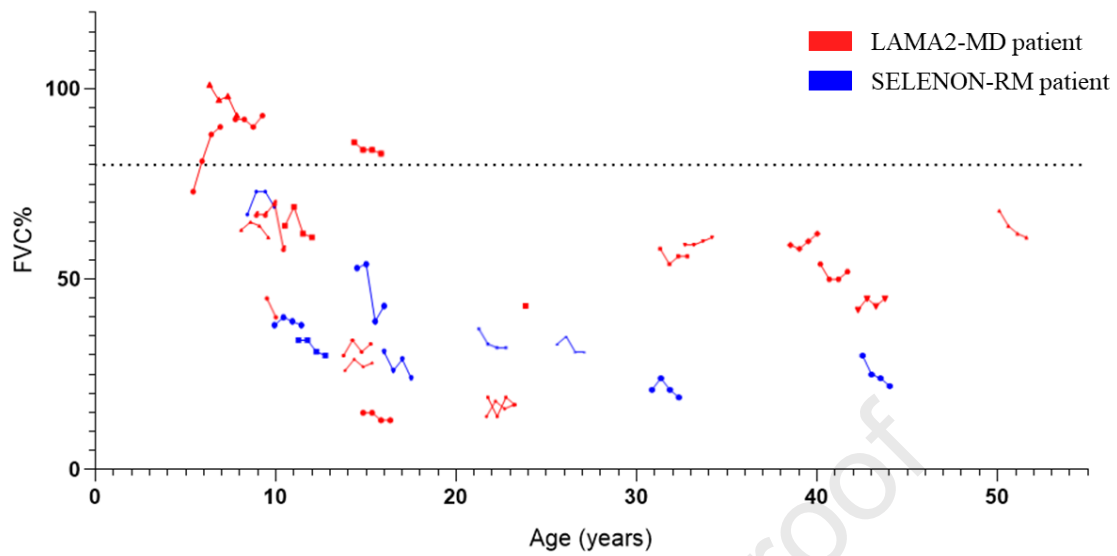
9 C7 and L2 and a Cobb's angle of 100 degrees between L2/L3. (c) severe thorax deformities showing pectus

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.

12

13



1

2

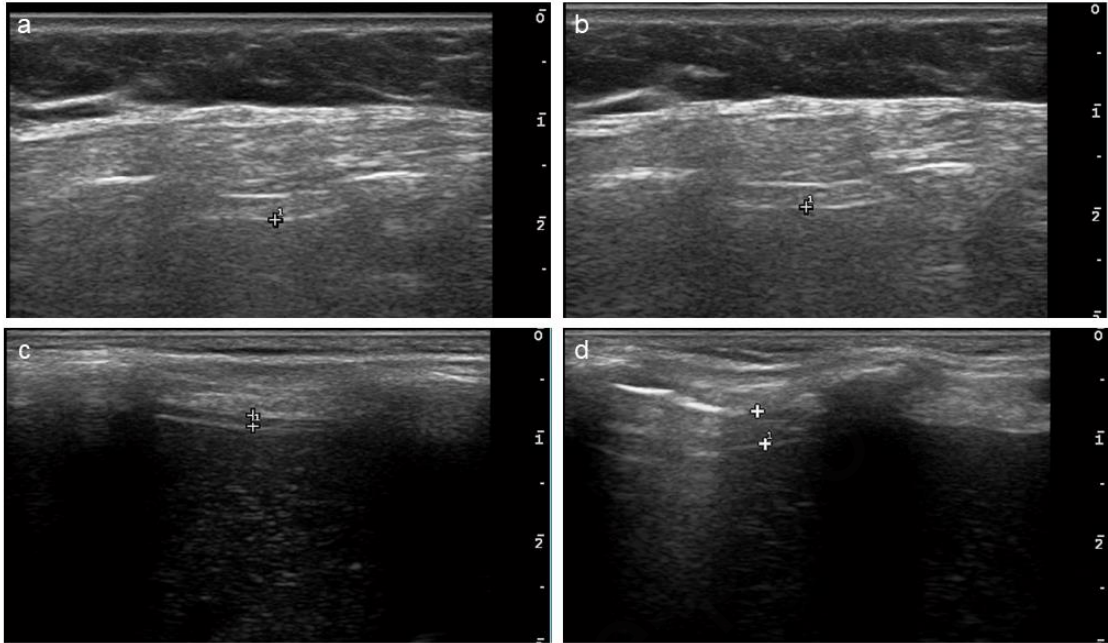
3 **Fig. 2 Overview on percentage predicted forced vital capacity (FVC%) in LAMA2-MD and SELENON-**

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.

6

1



2

3

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
LAMA2-MD		
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 ²)	21 [18; 24]	21 [18; 25]
BMI – child (kg/m ²)	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]
SELENON-RM		
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 ²)	23 [20; 25]	23 [21; 26]
BMI – child (kg/m ²)	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]

3

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
Spirometry					
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
Respiratory muscle strength					
	Baseline (n = 25)	6 months (n = 24)	12 months (n = 23)	18 months (n = 21)	
MIP (cmH ₂ O)	49 [29; 63]	43 [28; 58]	50 [25; 62]	52 [25; 64]	ns
MEP (cmH ₂ O)	29 [20; 52]	42 [21.; 68]	49 [25; 63]	45 [27; 66]	ns
SNIP (cmH ₂ O)	40 [28; 60]	40 [16; 60]	36 [23; 46]	48 [33; 66.5]	ns
Diaphragm ultrasound					
	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
Intercostal muscle ultrasound					
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

3

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
Spirometry					
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
Respiratory muscle strength					
	Baseline (n = 10)	6 months (n = 9)	12 months (n = 9)	18 months (n = 9)	
MIP (cmH ₂ O)	34 [26; 40]	34 [27; 47]	30 [25; 42]	27 [24.5; 41]	ns
MEP (cmH ₂ O)	50 [43; 62]	52 [37; 65]	50 [43; 65]	56 [43; 72]	ns
SNIP (cmH ₂ O)	37 [17; 44]	32 [22; 40]	31 [26; 37]	33 [21; 38]	ns
Diaphragm ultrasound					
	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.
Intercostal muscle ultrasound					
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].*

14

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 ₂ O)	MEP (mmH ₂ O)	SNIP (mmH ₂ 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*

3

4

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.*

9

1 **References**

2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50

1. Villar-Quiles RN, von der Hagen M, Méta y C, et al. The clinical, histologic, and genotypic spectrum of SEPN1-related myopathy: A case series. *Neurology*. 2020; 95: e1512-e1527.
2. Zambon AA, Ridout D, Main M, et al. LAMA2-related muscular dystrophy: Natural history of a large pediatric cohort. *Ann Clin Transl Neurol*. 2020; 7: 1870-1882.
3. Jain MS, Meilleur K, Kim E, et al. Longitudinal changes in clinical outcome measures in COL6-related dystrophies and LAMA2-related dystrophies. *Neurology*. 2019; 93: e1932-e1943.
4. Meilleur KG, Jain MS, Hynan LS, et al. Results of a two-year pilot study of clinical outcome measures in collagen VI- and laminin alpha2-related congenital muscular dystrophies. *Neuromuscul Disord*. 2015; 25: 43-54.
5. Bouman K, Groothuis JT, Doorduyn J, et al. LAMA2-Related Muscular Dystrophy Across the Life Span: A Cross-sectional Study. *Neurol Genet*. 2023; 9: e200089.
6. Oliveira J, Gruber A, Cardoso M, et al. LAMA2 gene mutation update: Toward a more comprehensive picture of the laminin- α 2 variome and its related phenotypes. *Hum Mutat*. 2018; 39: 1314-1337.
7. Ferreiro A, Quijano-Roy S, Pichereau C, et al. Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of multimimic disease: reassessing the nosology of early-onset myopathies. *Am J Hum Genet*. 2002; 71: 739-749.
8. Arbogast S, Beuvin M, Frayssé B, Zhou H, Muntoni F, Ferreiro A. Oxidative stress in SEPN1-related myopathy: from pathophysiology to treatment. *Ann Neurol*. 2009; 65: 677-686.
9. Arbogast S, Dill C, Ramahefasolo C, et al. *N-acetylcysteine as an effective treatment in vivo and identification of biomarkers in SEPN1-related myopathy: A first preclinical trial* 2014.
10. Smeitink J, van Maanen R, de Boer L, Ruitkamp G, Renkema H. A randomised placebo-controlled, double-blind phase II study to explore the safety, efficacy, and pharmacokinetics of sonlicromanol in children with genetically confirmed mitochondrial disease and motor symptoms ("KHENERGYC"). *BMC Neurology*. 2022; 22: 158.
11. Rooney JE, Knapp JR, Hodges BL, Wuebbles RD, Burkin DJ. Laminin-111 protein therapy reduces muscle pathology and improves viability of a mouse model of merosin-deficient congenital muscular dystrophy. *Am J Pathol*. 2012; 180: 1593-1602.
12. Kemaladewi DU, Bassi PS, Erwood S, et al. A mutation-independent approach for muscular dystrophy via upregulation of a modifier gene. *Nature*. 2019; 572: 125-130.
13. Kemaladewi D, Hyatt E, Ivakine Z, Cohn R. CRISPR/Cas9-mediated exon inclusion in Lama2 gene alleviates dystrophic pathology in MDC1A mouse model. *Neuromuscular Disorders*. 2016; 26: S190.
14. Wang CH, Bonnemann CG, Rutkowski A, et al. Consensus statement on standard of care for congenital muscular dystrophies. *J Child Neurol*. 2010; 25: 1559-1581.
15. Wang CH, Dowling JJ, North K, et al. Consensus statement on standard of care for congenital myopathies. *J Child Neurol*. 2012; 27: 363-382.
16. Sarkozy A, Foley AR, Zambon AA, Bönnemann CG, Muntoni F. LAMA2-Related Dystrophies: Clinical Phenotypes, Disease Biomarkers, and Clinical Trial Readiness. *Front Mol Neurosci*. 2020; 13: 123.
17. Meilleur KG, Linton MM, Fontana J, et al. Comparison of sitting and supine forced vital capacity in collagen VI-related dystrophy and laminin α 2-related dystrophy. *Pediatr Pulmonol*. 2017; 52: 524-532.
18. Caggiano S, Khirani S, Dabaj I, et al. Diaphragmatic dysfunction in SEPN1-related myopathy. *Neuromuscular Disorders*. 2017; 27: 747-755.
19. ATS/ERS Statement on respiratory muscle testing. *Am J Respir Crit Care Med*. 2002; 166: 518-624.

- 1 20. Graham BL, Steenbruggen I, Miller MR, et al. Standardization of Spirometry 2019 Update. An
2 Official American Thoracic Society and European Respiratory Society Technical Statement.
3 *Am J Respir Crit Care Med.* 2019; 200: e70-e88.
- 4 21. Oliveira J PFJ, Santos M, and Coelho T. LAMA2 Muscular Dystrophy. In: Adam MP, Feldman J,
5 Mirzaa GM, et al., editors. *GeneReviews*[®] [Internet]. Seattle (WA): University of Washington,
6 Seattle; 1993-2023. . 2012 Jun 7 [Updated 2020 Sep 17]; Available from:
7 <https://www.ncbi.nlm.nih.gov/books/NBK97333/>.
- 8 22. Park JJH, Siden E, Zoratti MJ, et al. Systematic review of basket trials, umbrella trials, and
9 platform trials: a landscape analysis of master protocols. *Trials.* 2019; 20: 572.
- 10 23. Bouman K, Groothuis JT, Doorduyn J, et al. Natural history, outcome measures and trial
11 readiness in LAMA2-related muscular dystrophy and SELENON-related myopathy in children
12 and adults: protocol of the LAST STRONG study. *BMC Neurol.* 2021; 21: 313.
- 13 24. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence
14 variants: a joint consensus recommendation of the American College of Medical Genetics
15 and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015; 17: 405-424.
- 16 25. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the
17 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J.* 2012; 40: 1324-
18 1343.
- 19 26. van Kleef ESB, van Doorn JLM, Gaytant MA, et al. Respiratory muscle function in patients
20 with nemaline myopathy. *Neuromuscular Disorders.* 2022.
- 21 27. Birnkrant DJ, Panitch HB, Benditt JO, et al. American College of Chest Physicians consensus
22 statement on the respiratory and related management of patients with Duchenne muscular
23 dystrophy undergoing anesthesia or sedation. *Chest.* 2007; 132: 1977-1986.
- 24 28. Laveneziana P, Albuquerque A, Aliverti A, et al. ERS statement on respiratory muscle testing
25 at rest and during exercise. *Eur Respir J.* 2019; 53.
- 26 29. van Doorn JLM, Wijntjes J, Saris CGJ, Ottenheijm CAC, van Alfen N, Doorduyn J. Association of
27 diaphragm thickness and echogenicity with age, sex, and body mass index in healthy
28 subjects. *Muscle Nerve.* 2022.
- 29 30. Horng MH, Kuok CP, Fu MJ, Lin CJ, Sun YN. Cobb Angle Measurement of Spine from X-Ray
30 Images Using Convolutional Neural Network. *Comput Math Methods Med.* 2019; 2019:
31 6357171.
- 32 31. Geranmayeh F, Clement E, Feng LH, et al. Genotype-phenotype correlation in a large
33 population of muscular dystrophy patients with LAMA2 mutations. *Neuromuscul Disord.*
34 2010; 20: 241-250.
- 35 32. Trundell D, Le Scouiller S, Le Goff L, Gorni K, Vuillerot C. Assessment of the validity and
36 reliability of the 32-item Motor Function Measure in individuals with Type 2 or non-
37 ambulant Type 3 spinal muscular atrophy. *PLoS One.* 2020; 15: e0238786.
- 38 33. Chabanon A, Seferian AM, Daron A, et al. Prospective and longitudinal natural history study
39 of patients with Type 2 and 3 spinal muscular atrophy: Baseline data NatHis-SMA study. *PLoS*
40 *One.* 2018; 13: e0201004.
- 41 34. Teeselink S, Vincenten SCC, Voermans NC, et al. Long-term follow-up of respiratory function
42 in facioscapulohumeral muscular dystrophy. *Journal of Neurology.* 2022; 269: 3682-3689.
- 43 35. Mayer OH. Scoliosis and the impact in neuromuscular disease. *Paediatr Respir Rev.* 2015; 16:
44 35-42.
- 45 36. Fromageot C, Lofaso F, Annane D, et al. Supine fall in lung volumes in the assessment of
46 diaphragmatic weakness in neuromuscular disorders. *Arch Phys Med Rehabil.* 2001; 82: 123-
47 128.
- 48 37. Wilding RJ, Thynne M, Subhan MMF. Optimization of sniff nasal inspiratory pressure (SNIP)
49 measurement methodology in healthy subjects. *BMC Pulmonary Medicine.* 2023; 23: 66.
- 50 38. Prigent H, Orlikowski D, Fermanian C, et al. Sniff and Muller manoeuvres to measure
51 diaphragmatic muscle strength. *Respiratory Medicine.* 2008; 102: 1737-1743.

- 1 39. Silwal A, Sarkozy A, Scoto M, et al. Selenoprotein N-related myopathy: a retrospective
2 natural history study to guide clinical trials. *Ann Clin Transl Neurol.* 2020; 7: 2288-2296.
- 3 40. van Doorn JLM, Pennati F, Hansen HHG, van Engelen BGM, Aliverti A, Doorduyn J.
4 Respiratory muscle imaging by ultrasound and MRI in neuromuscular disorders. *Eur Respir J.*
5 2021; 58.
- 6

Journal Pre-proof

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

Journal Pre-proof

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
Peter J. Wijkstra	none
Baziel G.M. van Engelen	none
Corrie E. Erasmus	none
Jonne Doorduyn	none
Nicol C. Voermans	none

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